PHYLOGENETICS OF THE GENUS *Scotophilus* (CHIROPTERA:VESPERTILIONIDAE): PERSPECTIVES FROM PATERNALLY AND MATERNALLY INHERITED GENOMES WITH EMPHASIS ON AFRICAN SPECIES

A Dissertation

by

ROBERT GREG TRUJILLO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2005

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

Chair of Committee,	John W. Bickham
Committee Members,	Rodney L. Honeycutt
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	Duane A. Schlitter
Head of Department,	Robert D. Brown

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ABSTRACT

Phylogenetics of the Genus *Scotophilus*

(Chiroptera: Vespertilionidae): Perspectives from Paternally and Maternally Inherited Genomes with Emphasis on African Species. (August 2005)

Robert Greg Trujillo, B.S., University of New Mexico;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. John W. Bickham

Bats of the genus *Scotophilus* are distributed throughout sub-Saharan Africa, parts of southern and Southeast Asia, a majority of the Indomalayan Islands, Reunion Island, and Madagascar. The genus is composed of 14 recognized species with seven distributed throughout sub-Saharan Africa including: (*S. dinganii* (A. Smith, 1833), *S. leucogaster* (Cretzschmar, 1830), *S. nigritellus* de Winton, 1899, *S. nigrita* (Schreber, 1774), *S. nucella* Robbins, 1983, *S. nux* Thomas, 1904, and *S. viridis* (Peters, 1852). The remaining species include four from southern and southeast Asia (*S. celebensis* Sody, 1928; *S. collinus* Sody 1936; *S. heathi* (Horsfield, 1831); *S. kuhlii* Leach, 1821), two on Madagascar (*S. sp. nov*. Goodman et al., in press; and *S. robustus* Milne-Edwards, 1881), and one endemic to Reunion Island (*S. borbonicus* (E. Geoffroy, 1803).

The systematics and taxonomy of this genus have been controversial and continue to be confusing. The genus is plagued with problems in species definition and the systematic relationships among members of the genus are poorly understood. The major goal of this study was to use a molecular phylogenetic approach to clarify some of the controversy and confusion surrounding the members of this genus.

Nucleotide differences from mtDNA and the Y chromosome were used to examine phylogenetic patterns within *Scotophilus*. Based on these data two new species of *Scotophilus* were identified. Phylogenetically, African *Scotophilus* were found to comprise a monophyletic group with *S. nux* as the most basal African taxon. Overall, the Asian *S. kuhlii* was the most basal taxon. A distant relationship was identified between *S. kuhlii* and *S. heathi*, the other Asian species examined. The multiple origins of Malagasy *Scotophilus* are apparent as the two Malagasy taxa in the study do not share a sister-group relationship. The large bodied *S. nigrita* is closely related to *S. dinganii* and the *S. dinganii*-like species all share a close relationship. *S. nigrita* has a *S. dinganii*-like mtDNA haplotype and a very distinct *zfy* haplotype, suggesting a possible hybridization event with a *S. dinganii*-like ancestor.

DEDICATION

This dissertation is dedicated to my late maternal grandfather, Jose Alfonso Martinez. It is through his examples and teachings that I have developed the work ethic that has been the reason for my continued academic success.

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The successful completion of a graduate program is rarely due to the work of one single individual but is rather the result of the influence and support of a large circle of friends and colleagues. The successful completion of my doctoral program can be characterized by the previous statement as there are many people who have contributed and influenced me throughout my graduate career. The words I write in this section can only begin to show my gratitude.

I would like to acknowledge my committee chair for his continual support and encouragement. Dr. John W. Bickham is the kind of advisor most graduate students would love to have. He has constantly found ways to support me in the lab and has supported my scholarly endeavors no matter how far fetched they may seem. Because of his support, I have been able to pursue many research projects outside of my dissertation work and have even participated in professional development activities that have demanded time away from my research. It has been a pleasure and an honor to be a graduate student in Dr. Bickham's lab and I express my sincere thanks for everything you have done for me.

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I would like to acknowledge the many scientists and museums that contributed tissues to this study. Tissues included in this study were kindly provided by Jim Patton of the Museum of Vertebrate Zoology, Judith Eger of the Royal Ontario Museum, Steve Goodman and Lawrence Heaney of the Field Museum of Natural History, Sue Mclaren of the Carnegie Museum Section of Mammals, and Zubaid Akbar of the University Kebangsaan in Malaysia. I would like to thank Dr. Duane Schlitter for his tireless efforts in collecting the small mammals of Africa. It is Dr. Schlitter who was responsible for the collection of over 70% of the specimens used in this study. I would also like to thank Steve Goodman, Judith Eger, and Sue Mclaren for comments on and identification of specimens included in this study. The value of scientific collections can not be emphasized enough and I only hope I will be able to repay the contribution of these tissues to my study through the collection and deposition of voucher specimens in

museum collections. My thanks also go out to the field crews that collected these specimens as I have experienced firsthand the rigors of small mammal surveys.

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I would like to acknowledge the members of the Bickham lab for all of their support and encouragement. Dr. John Patton has had a significant influence on this project and I am indebted to him for the use of the *zfy* primers he developed as well as for the many conversations we have had over the last several years. Ryan Huebinger has been a good friend and has provided me many an opportunity to leave the stress of graduate school behind in exchange for fishing or hunting adventures. Dr. Cole Matson has provided his technical expertise and has demonstrated to me the proper way to use Microsoft word on numerous occasions.

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Words can not easily express the gratitude I wish to convey to my wife, Adriana, and to my boys, Joseph and Matthew. All three of you have sacrificed so much for me to complete graduate school and I am forever indebted to each of you. Adriana has tirelessly supported our family while I have been a graduate student. I will never forget your hard work and encouragement. Joseph and Matthew are the greatest sons a father could ever ask for. I am extremely proud of both of you and thankful for your presence in my life.

I also owe a word of thanks to Dr. Duane and Judy Schlitter who have been an adopted set of grandparents to my kids. Joseph and Matthew's eyes light up when they find out we are visting Dr. Duane and Mrs. Judy. I am certainly speaking for my family when I say you two have greatly enriched our lives.

Alex and Veronica Navarro have also been dear friends and have supported me and my family in any way they can. I wish Alex and Veronica the best in their future and thank them for their friendship.

I would like to acknowledge the support and encouragement provided by my in-laws, Rudolfo and Margarita Sanchez. My in-laws have helped us out in many ways and I am thankful for this support.

NOMENCLATURE

mtDNA	mitochondrial DNA
zfy	y restricted zinc finger gene in mammals
bp	base pair
CM	Carnegie Museum of Natural History
FMNH	The Field Museum of Natural History
TM	Transvaal Museum
ROM	Royal Ontario Museum
MVZ	Museum of Vertebrate Zoology
UADBA	Université d'Antananarivo, Département de Biologie Animale

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INTRODUCTION

Bats of the Genus *Scotophilus*

The genus *Scotophilus* (Leach, 1821) occurs throughout sub-Saharan Africa, parts of southern and Southeast Asia, a majority of the Indomalayan Islands, Reunion Island, and Madagascar. The genus is a member of the family Vespertilionidae, subfamily Vespertilioninae, and tribe Scotophilini (Hoofer and Van Den Busche, 2003). Currently, Simmons (in press) recognizes 12 species of *Scotophilus* in the latest version of Mammal Species of the World 3rd Edition including: *S. borbonicus* (E. Geoffroy, 1803), *S. celebensis* Sody, 1928, *S. collinus* Sody 1936, *S. dinganii* (A. Smith, 1833), *S. heathi* (Horsfield, 1831), *S. kuhlii* Leach, 1821, *S. leucogaster* (Cretzschmar, 1830), *S. nigrita* (Schreber, 1774), *S. nucella* Robbins, 1983 , *S. nux* Thomas, 1904, *S. robustus* Milne-Edwards, 1881, and *S. viridis* (Peters, 1852). Another species, *S. nigritellus* de Winton, 1899, is recognized by Grub et al. (1998). Seven species occur in Africa (*S. dinganii*, *S. leucogaster*, *S. nigrita*, *S. nigritellus, S. nux*, *S. nucella,* and *S. viridis*), one is endemic to Madagascar (*S. robustus*), one endemic to Reunion Island (*S. borbonicus*), one is endemic to the Indonesian island of Sulawesi (*S. celebensis*), two occur throughout India and Southeast Asia (*S. heathi* and *S. kuhlii*), and one occurs on the Indonesian islands of Java and Bali and was recently identified as a distinct species (*S. collinus*, Kitchener et al., 1997). Of the African species, *S. leucogaster* has been recorded outside Africa from two locations, one in extreme southwestern Saudi Arabia

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This dissertation follows the style of Systematic Biology.

from a location on the Abha-Raydah escarpment (Gaucher, 1993) and another from Yemen (Al-Safadi, 1991).

Members of *Scotophilus* have been shown to comprise a monophyletic group based on several lines of evidence. From a morphological standpoint, several features appear to be diagnostic for the genus, including a less prominent W-shaped molar cusp pattern with internal legs poorly developed (Rosevear, 1965), a single pair of large upper incisors with a dental formula of 1/3 1/1 1/2 3/3 = 30 (Dobson, 1875), and several distinct features of the baculum (Hill and Harrison, 1987). Hill and Harrison (1987) concluded that *Scotophilus* was sufficiently distinct from all other vespertilionines and these differences warranted tribal status. They further noted that the genus *Scotomanes* possesses several bacular similarities with *Scotophilus* and included both *Scotophilus* and *Scotomanes* in the tribe Scotophilini (Hill and Harrison, 1987). However, a recent study by Hoofer and Van Den Bussche (2003) based on mitochondrial 12s rRNA, $tRNA^{Val}$, and 16s rRNA sequences did not find any support for a close relationship between *Scotomanes* and *Scotophilus*, but the level of divergence between *Scotophilus* and all other vespertilionines is sufficient to warrant tribal status. Hoofer and Van Den Bussche (2003) also find support for the monophyly of *Scotophilus* based on their genetic data and proposed *Scotophilus* as the sole member of Scotophilini.

Several studies have enhanced our understanding of the biology of various species of *Scotophilus*. The common name for the genus *Scotophilus* is house bat or yellow bat. As the name implies, these bats are known to roost in roofs, attics, and walls of houses (Rosevear, 1965; Lekagul and McNeeley, 1977) as well as in thatched roofs

(Kingdon, 1974). Thus, these bats are usually found in close association with human activity (Nowak and Paradiso, 1983), and are known to use tree hollows (Fenton, 1983) and fan palm fronds (Rickart et al., 1989) as roosts. Like most vespertilionids, bats of the genus *Scotophilus* are insectivorous with a diet consisting mainly of beetles, termites, and moths along with other insects (Nowak and Paradiso, 1983; Barclay, 1985). Individuals of *S. leucogaster* were found to forage primarily over floodplains for a period of less than one hour at dusk while spending the remainder of the night in tree hollow night roosts (Barclay, 1985). Members of *Scotophilus* are characterized by strong flight and heavy dentition (Kitchener et al., 1990) with both characteristics necessary for their food habits.

The systematics and taxonomy of this genus have been controversial and continue to be confusing. A brief history of the taxonomy and systematics of the genus follows after a short description of the species recognized in this study. The seven recognized African species are distinguishable based on their morphology. *S. nigrita* is larger than all other African *Scotophilus* (Koopman 1975, 1994; Rosevear 1965), and differences among the other six African species are related to both size and pelage color. Both *S. dinganii* and *S. viridis* have a yellow-tinged ventral pelage with *S. dinganii* being the larger of the two. *S. nigritellus* is distributed in western Africa and is morphologically similar to *S. viridis*. *S. leucogaster* has a white to grey ventral pelage, and *S. nux* is characterized by a dark brown pelage on the dorsal side and a lighter brown pelage on the ventral sides (Robbins et al., 1985). According to Robbins (1983), *S. nucella* is distinct from both *S. nux* and *S. leucogaster* and is restricted to the high forest

vegetation zone in Ghana and Uganda. *S. nucella* is distinguished from *S. nux* by its smaller skull, wing and body measurements and from *S. leucogaster* by a more inflated brain case, a more developed sagittal crest, and a more projecting helmet (Robbins, 1983). The Reunion Island species, *S. borbonicus*, is presumably known only from a single lectotype specimen (Hill, 1980). The specimen is in poor condition but nonetheless has a dull white ventral pelage with a light reddish brown dorsal pelage and, although faded, agrees with the original description given for this specimen (Hill, 1980). The Madagascan species, *S. robustus*, is characterized by a short rostrum, a well developed sagittal crest, and its large size (Peterson et al., 1995), but none of the other species approach *S. nigrita* in large size. One of the Asian species, *S. heathi*, has small ears, a broad blunt muzzle, and a fine pelage that is a pale yellow throughout the throat, chest, and belly and darker above but variable with geographic range (Bates and Harrison, 1997). Another species distributed throughout southern and Southeast Asia, *S. kuhlii*, is described as being smaller than *S. heathi* with a brownish pelage above and paler through the throat, chest, and belly without the yellow tinge seen in *S. heathi* (Bates and Harrison, 1997). A recently recognized species, *S. collinus*, is described as slightly smaller than *S. kuhlii* with a brown dorsum and paler and yellowish venter (Kitchener et al., 1997). The Sulawesi endemic, *S. celebensis*, is morphologically similar to *S. heathi*.

Taxonomy and Systematics

The genus *Scotophilus* was first described by Leach (1821) based on a single immature specimen that still retained its deciduous milk teeth (Dobson, 1875). This

specimen became the type specimen of *S. kuhlii*. Dobson (1875) questioned whether the genus should be rejected due to its founding on a specimen whose species it belongs to can not be accurately determined. The very beginnings of *Scotophilus* taxonomy are surrounded in confusion and as Dobson (1875) points out, at the time English and American zoologists applied the name *Scotophilus* to almost every bat from the family Vespertilionidae with less than 38 teeth.

The genus *Scotophilus* has continued to have a complex and controversial taxonomic history and several systematic issues remain unresolved. Several of the issues, both past and present, include: 1) the affinity of *S. borbonicus* from Reunion Island with any of the smaller African mainland species; 2) the number of species and names of species present in the southern African subregion as well as in the Ethiopian subregion; 3) the occurrence of *S. borbonicus* on Madagascar; 4) the affinity of Madagascan species with African mainland species; 5) confusion over the name of the largest African species (*S. nigrita*); and 6) the phylogenetic and phylogeographic relationships of all members of the genus.

The largest African species, *S. nigrita*, was referred to as *S. gigas* in the past, while *S. nigrita* was used to refer to what is now *S. dinganii*. Robbins (1978) analyzed the taxonomic identification and history of *S. nigrita* and found that *S. nigrita* was the senior synonym of *S. gigas*. Therefore, what was referred to as *S. nigrita* in the literature for years is now *S. dinganii*, and *S. gigas* became a junior synonym of *S. nigrita*.

The number of species present in southern Africa has been a controversial topic with uncertainty still remaining. A very thorough morphometric overview of African

Scotophilus taxonomy and systematics is provided by Robbins et al. (1985) and more recently was summarized by Bronner et al. (2003). I will highlight the major contentions only. Ellerman et al. (1953) recognized three species occurring in the southern African subregion: *S. gigas* [=*nigrita*], *S. nigrita* [=*dinganii*], and *S. viridis* as distinct from Reunion Island *S. borbonicus*. Hill (1980) considered *S. borbonicus* conspecific with *S. viridis* of southern Africa as did Koopman (1984, 1994), and both recognized two subspecies of *S. borbonicus*: *S. b. borbonicus* on Reunion Island, which may be extinct (Cheke and Dahl, 1981), and *S. b. viridis* on mainland southern Africa. Meester et al. (1986) recognized the same species present in southern Africa as Hill (1980) and Koopman (1984, 1994). Initially, Koopman (1975) restricted *S. borbonicus* to Madagascar and Reunion Island. Corbet and Hill (1980) followed Koopman (1975) in regards to *S. borbonicus* and recognized *S. viridis* as a distinct species. This arrangement was followed by Swanepoel et al. (1980) as well. Robbins et al. (1985) rejected the use of *S. borbonicus* for either species or subspecies on the African mainland and recognized *S. viridis* as a distinct African mainland species with a distribution from Senegal east to Ethiopia and south to southeastern South Africa. Robbins et al. (1985) recognized four species in southern Africa: *S. nigrita*, *S. dinagnii*, *S. leucogaster*, and *S. viridis* (also as distinct from *S. borbonicus*).

The most comprehensive morphometric study to date on African *Scotophilus* is that of Robbins et al. (1985), who examined over 2000 specimens from throughout sub-Saharan Africa including 11 type specimens in their analysis. Robbins et al. (1985) recognized six species of *Scotophilus* occurring in sub-Saharan Africa: *S. dinganii*, *S.*

leucogaster, *S. nigrita*, *S. nucella*, *S. nux*, and *S. viridis*. They list *S. nigritellus* as a synonym of *S. viridis* but note that specimens from western Africa differ from *S. viridis* from east and southern Africa (Robbins et al., 1985). This study serves as the basis for the current taxonomy of *Scotophilus* and is followed by several authors (Bronner et al., 2003; Simmons, in press; Nowak, 1999 as well as others). Robbins et al. (1985) identified the species present in several areas of Africa as follows: West African savannah sympatric species (*S. dinganii*, *S. leucogaster*, and *S. viridis*), Savannah species occurring from west to east Africa but not occurring sympatrically (*S. dinganii*, *S. leucogaster*, and *S. viridis*), species from the high forest occurring from west to east Africa (*S. dinganii* and *S. nux*), and the four species from southern Africa with the southern African species of *S. viridis* being slightly larger and showing more variation than in other parts of Africa (*S. nigrita*, *S. dinagnii*, *S. leucogaster*, and *S. viridis*).

The other *Scotophilus* found in Africa, *S. nucella*, were recognized by Robbins (1983) as being distinct from *S. nux* and *S. leucogaster*, and are restricted to the high forest vegetation zone in Ghana and Uganda. This species is listed as a synonym of *S. leucogaster* in *Mammal Species of the World 2nd Edition* (Koopman, 1993) although Robbins (1983) clearly separated *S. nucella* from *S. leucogaster* through discriminant analysis utilizing wing and cranial measurements. Simmons (in press) recognized *S. nucella* as a valid species.

Madagascan species of *Scotophilus* have also not been left out of the controversy. The Reunion Island species, *S. borbonicus*, has been reported as present in Madagascar (Dorst, 1974) and this designation has been followed by Hill (1980), Nowak and

Paradiso (1983), Robbins et al. (1985), Nowak (1999), and Koopman (1993, 1994). Peterson et al. (1995) note that they studied no specimens of *S. borbonicus* from Madagascar in all the Malagasy specimens they examined. Goodman et al. (in press) examined some specimens of a small *Scotophilus* collected on Madagascar and concluded that given the condition of the Reunion Island lectotype (partially shattered cranium) they could not make several measurements, thus precluding a decision as to the status of *S. borbonicus* on Madagascar. They referred to one of these specimens as *S. cf. borbonicus* and described the other as a new species previously unknown to science (Goodman et al., in press). The Reunion Island *S. borbonicus* has not been collected in recent years and is presumed to be extinct (Cheke and Dahl, 1981). In regards to *S. robustus*, Koopman (1994) considered *S. robustus* as a subspecies of *S. leucogaster*, but Robbins et al. (1985) considered *S. robustus* as a distinct species as does Simmons (in press).

Hoofer and Van Den Bussche (2003) examined seven species of *Scotophilus*. The molecular data presented by Hoofer and Van Den Bussche (2003) revealed a close relationship among four Ethiopian species (*S. borbonicus* [=*S. viridis*], *S. dinganii*, *S. leucogaster*, and *S. nux*) and a distant relationship between two Indomalayan species (*S. heathi* and *S. kuhlii*), although the authors did not provide conclusions as to the systematics of *Scotophilus* citing the unreliable and confused taxonomy of *Scotophilus*. The authors do conclude that their data supported the monophyly of *Scotophilus* and that the sequence divergence between all of the specimens was representative of species-level comparisons (Hoofer and Van Den Bussche, 2003).

Justifying the Y Chromosome

The majority of molecular systematic projects, both in the recent past and currently, are based on the study of mitochondrial DNA (mtDNA). Utility of mtDNA as a population and phylogenetic marker is well established with certain regions of the mitochondrial genome suitable for population level studies (see Bickham et al., 1996; Trujillo et al., 2004; as examples of the use of the mtDNA control region to investigate the population genetics of Steller's sea lions), while other regions have been demonstrated to effectively recover species phylogenies (Bradley and Baker, 2001). The merits of mtDNA markers for use in molecular systematics are detailed by Avise (2004).

Confirmation of mtDNA-based phylogenies by nuclear markers has become the most recent trend in molecular systematics. Examples of this approach include studies on the genus *Mus* (Lundigran et al., 2002; Tucker et al., 2003), cercopithecine monkeys (Tosi et al., 2003), equids (Wallner et al., 2003), the order Chiroptera (Teeling et al., 2000; 2002; 2005), and the class Mammalia (Murphy et al., 2001) to name a few. Benefits of such an approach are obvious as data from nuclear markers will either corroborate mtDNA based phylogenies or provide new phylogenetic hypotheses for testing.

In particular, the Y chromosome has been suggested as a molecular marker suitable for population genetic studies as well as for phylogenetic studies. In humans, the Y chromosome has a long history of use as a molecular marker and a review of the human Y chromosome and its applications is given by Jobling and Tyler-Smith (2003).

For specific studies on the use of the human Y chromosome see Semino et al. (2002) or Bosch et al. (2001).

The *zfy* gene encodes a zinc finger protein and is restricted to the mammalian Y chromosome. The *zfy* gene is located outside of the pseudoautosomal region on the p arm of the Y chromosome (Page et al., 1987; Mardon and Page, 1989). It is autosomal in marsupials (Sinclair et al., 1988) and monotremes (Watson et al., 1993). The *zfy* gene is found located in the sex chromosomes in Rodentia (Bianchi et al., 1992), carnivores (Lanfear and Holland, 1991), and primates (Palmer et al., 1990).

A study on the genus *Equus* found fixed differences in Y chromosome sequences between various species of *Equus* (Wallner et al., 2003). A 1,887 bp segment of the Y chromosome was examined, with between 2 and 32 changes occurring among various equid species (representing 0.107% - 1.734% sequence divergence between species; Wallner et al., 2003). This study also included two subspecies of zebra with a 1 bp change representing 0.053% sequence divergence between the two. This low level of intraspecific sequence variability is comparable to low levels observed in other mammalian species as reported by Shen et al. (2000) and Hellborg and Ellegren (2004).

A study by Slattery and O'Brien (1998) demonstrated that sequences of an intron of the *zfy* gene accurately tracked hierarchical topologies among species of cats (Felidae). They note that the Y chromosome demonstrated a "remarkable degree of phylogenetic consistency", and concluded that *zfy* is "highly accurate in recapitulating evolutionary history". Another study using sequences of the final intron of *zfy* was that of Lawson and Hewitt (2002), which investigated substitution rates in *zfx* and *zfy* introns

in sheep and goats. They reported a range of 0.777% to 6.38% sequence divergence between genera and a range of 0.129% to 0.648% between species (Lawson and Hewitt, 2002). The between species range was comparable to that reported by Wallner et al. (2003) for equids. In general, the *zfy* gene seems to be a suitable marker for inferring species level phylogenies and to compare phylogenies derived from mtDNA.

The *zfy* gene represents a haploid genome transferred from father to son, does not undergo recombination, and has been demonstrated to accurately infer phylogenies. It is for these reasons that a Y chromosome marker, particularly *zfy*, has been chosen to infer the phylogeny of bats of the genus *Scotophilus* in conjunction with the cytochrome *b* gene of the mitochondrial genome.

Study Objectives

Three objectives of this study are as follows: (1) conduct a phylogenetic analysis of the genus *Scotophilus*, elucidate the relationships between species, and determine the number of species based on mitochondrial DNA and Y chromosome markers with special emphasis on the African species, (2) investigate the level of concordance between a mitochondrial and a Y chromosome data set and evaluate the usefulness of a Y chromosome marker in phylogeny reconstruction, and (3) investigate the phylogeographic history of the genus *Scotophilus*.

In this study I will assess the phylogenetic relationships among the various taxa and use the resulting phylogenies to examine the phylogeography of the group with special emphasis on the African radiation. Specifically, I will test the hypothesis of monophyly of the African species. Furthermore, I will attempt to determine the

geographic area of origin of the Madagascan species and whether or not there has been a single invasion or multiple invasions either into Africa or Asia. I will also attempt to determine if there are any clear biogeographic tracks within Africa. Do multiple species from specific areas appear to comprise monophyletic groups relative to species from other areas? Finally, I will compare the relative utilities of maternally inherited (mtDNA) and paternally inherited (Y-chromosome) molecular markers. In particular I will look for patterns of non-concordance between the two markers that are possibly due to real evolutionary processes, and not reflective of problems with resolution. Such processes might include interspecific hybridization (Cathey et al. 1998) and/or other events that can lead to differences between gene trees and species trees.

Several taxonomic issues will be addressed. Within the group of *S. viridis* present in Africa, I will determine if this group represents one species or if there is sufficient evidence for the validity of multiple small body species on mainland Africa. Also, I will investigate the widespread *S. dinganii* in terms of genetic divergence between populations from east, west, and southern Africa. The position of *S. nigrita* on the inferred phylogeny will also help to resolve any confusion that exists in relation to this large *Scotophilus* and other members of the genus. I will determine a center of origin for the Malagasy *Scotophilus* and identify the sister taxa to other Malagasy species. Unfortunately, I will be unable to investigate the relationship between *S. nucella*, *S. collinus*, and *S. celebensis* to other members of the genus. I will also be unable to resolve the issue of whether the type locality of *S. borbonicus* represents a source for mainland African *S. viridis*. The results of this study should provide several

testable hypotheses as to the systematic relationships between members of the genus *Scotophilus* and help to sort out some of the confusion that has plagued this genus. To my knowledge, this is the first extensive molecular study of the genus *Scotophilus*, and the results should prove invaluable in addressing various issues associated with the genus.

METHODS

Study Area and Sample Size

In this study, 137 specimens were examined representing 10 species of *Scotophilus* from ten countries. Figure 1 shows the approximate sampling localities of the specimens examined. Specimens examined, including catalog numbers and localities, are listed in table 1.

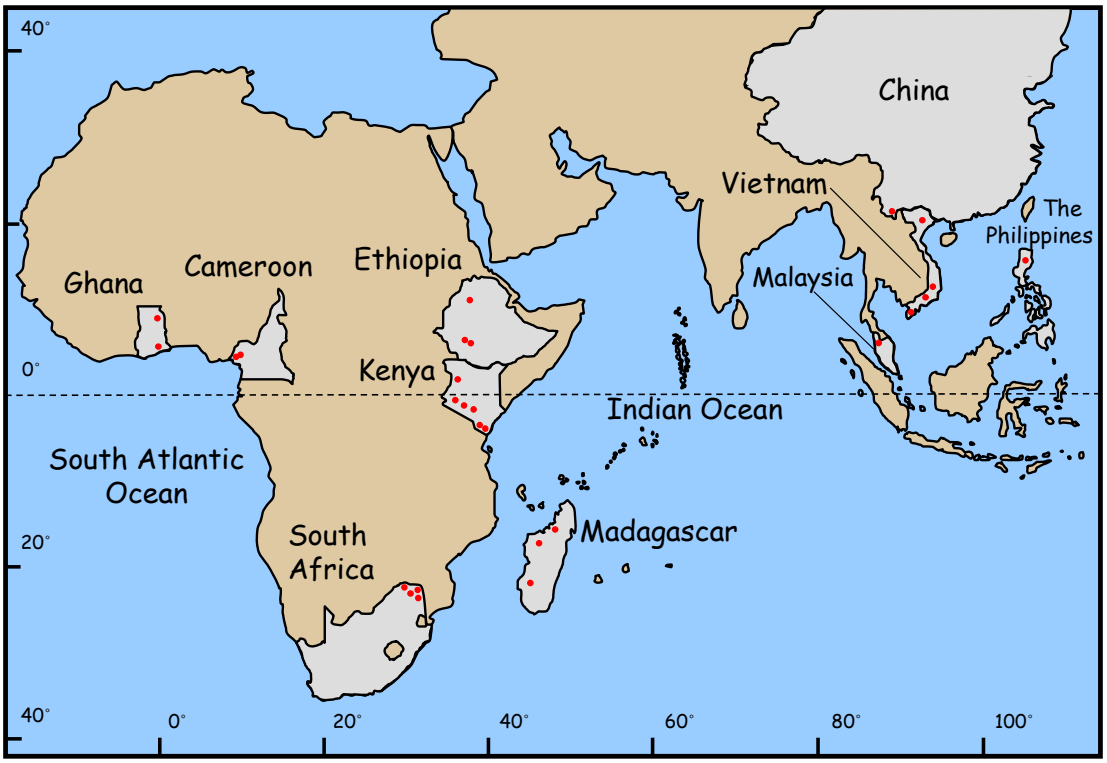


Figure 1. Sampling localities of *Scotophilus*.

Table 1. Specimens examined in this study.

Species	Tissue Collection Number	Museum Catalog Number	Locality
S. dinganii	SP 5454	CM 102245	Kenya: Coast Province: Taita District
S. dinganii	SP 5453	CM 102244	Kenya: Coast Province: Taita District
S. dinganii	SP 5078	CM 102252	Kenya: Western Province: Kakamega District
S. dinganii	SP 5368		Kenya: Rift Valley Province: West Polcot District
S. dinganii	SP 5052	CM 102251	Kenya: Western Province: Kakamega District
S. dinganii	SP 5452		Kenya: Coast Province: Taita District
S. dinganii	SP 5451		Kenya: Coast Province: Taita District
S. dinganii	SP 5051	CM 102253	Kenya: Western Province: Kakamega District
S. dinganii	SP 5077		Kenya: Western Province: Kakamega District
S. dinganii	SP 5082	CM 102253	Kenya: Western Province: Kakamega District
S. dinganii	SP 5084	CM 102255	Kenya: Western Province: Kakamega District
S. dinganii	SP 5370	CM 102247	Kenya: Rift Valley Province: West Polcot District
S. dinganii	SP 5386	CM 102249	Kenya: Rift Valley Province: West Polcot District
S. dinganii	TK 33149	CM 98050	Kenya: Rift Valley Province: Nakuru District
S. dinganii	TK 33395	CM 98057	Kenya: Eastern Province: Machakos District
S. dinganii	TK 33534	CM 98051	Kenya: Western Province: Kakamega District
S. dinganii	SP 5083	CM 102254	Kenya: Western Province: Kakamega District
S. dinganii	TK 33142		Kenya: Rift Valley Province: Nakuru District
S. dinganii	TK 33141		Kenya: Rift Valley Province: Nakuru District
S. dinganii	SP 5385	CM 102248	Kenya: Rift Valley Province: West Polcot District
S. dinganii	SP 5369	CM 102246	Kenya: Rift Valley Province: West Polcot District
S. dinganii	TK 33359	CM 98054	Kenya: Eastern Province: Machakos District
S. dinganii	TK 33360	CM 98045	Kenya: Eastern Province: Machakos District

Table 1. continued

Species	Tissue Collection Number	Museum Catalog Number	Locality
S. dinganii	TK 33361	CM 98044	Kenya: Eastern Province: Machakos District
S. dinganii	TK 33535	CM 98052	Kenya: Western Province: Kakamega District
S. dinganii	TK 33536	CM 98053	Kenya: Western Province: Kakamega District
S. dinganii	TK 33140	CM 98048	Kenya: Rift Valley Province: Nakuru District
S. dinganii	TK 33189	CM 98043	Kenya: Coast Province: Kwale District
S. dinganii	SP 13027	CM 114043	Ethiopia: Gondar Province
S. dinganii	AK 21213	uncataloged	Ethiopia: Oromiya Region: Dogy River Bridge
S. dinganii	AK 21234	uncataloged	Ethiopia: Oromiya Region: Dogy River Bridge
S. dinganii	AK 21215	uncataloged	Ethiopia: Oromiya Region: Dogy River Bridge
S. dinganii	AK 21235	uncataloged	Ethiopia: Oromiya Region: Dogy River Bridge
S. dinganii	AK 21214	uncataloged	Ethiopia: Oromiya Region: Dogy River Bridge
S. dinganii	AK 21259	uncataloged	Ethiopia: Oromiya Region: Dogy River Bridge
S. dinganii	AK 21223	uncataloged	Ethiopia: Oromiya Region: Dogy River Bridge
S. dinganii	AK 21163	uncataloged	Ethiopia: Gambela Region: Bishen Waca Lake
S. dinganii	TM 38151	TM 38151	South Africa
S. dinganii	TM 37655	TM 37655	South Africa: Transvaal Province: Pafuri district
S. dinganii	TM 37656	TM 37656	South Africa: Transvaal Province: Pafuri district
S. dinganii	TM 37668	TM 37668	South Africa: Transvaal Province: Pafuri district
S. dinganii	TM 37669	TM 37669	South Africa: Transvaal Province: Pafuri district
S. dinganii	SP 7731	CM 105747	South Africa: Transvaal Province: Fram Greefswald 37
S. dinganii	SP 7732	CM 105748	South Africa: Transvaal Province: Fram Greefswald 37
S. dinganii	SP 7755	CM 105749	South Africa: Transvaal Province: Fram Greefswald 37
S. dinganii	SP 7789	CM 105750	South Africa: Transvaal Province: Fram Greefswald 37

Table 1. continued

Species	Tissue Collection Number	Museum Catalog Number	Locality
S. dinganii	TM 39625	TM 39625	South Africa: Mpumalanga Province: Satara
S. dinganii	TM 39624	TM 39624	South Africa: Mpumalanga Province: Satara
S. dinganii	F 52131	uncataloged	South Africa
S. dinganii	SP10179	CM 113641	Ghana: Greater Accra Region
S. dinganii	SP10180	CM 113642	Ghana: Greater Accra Region
S. dinganii	SP10181	CM 113643	Ghana: Greater Accra Region
S. leucogaster	SP10136	CM 113645	Ghana: Northern Region
S. leucogaster	SP10137	CM 113646	Ghana: Northern Region
S. nigrita	SP 5505	CM 102256	Kenya: Coast Province: Taita District
S. nux	SP 5053	CM 102257	Kenya: Western Province: Kakamega District
S. nux	SP 5056	CM 102260	Kenya: Western Province: Kakamega District
S. nux	TK 33485		Kenya: Western Province: Kakamega District
S. nux	SP 5054	CM 102258	Kenya: Western Province: Kakamega District
S. nux	TK 33519	CM 98056	Kenya: Western Province: Kakamega District
S. nux	SP 5055	CM 102259	Kenya: Western Province: Kakamega District
S. nux	SP 10620	CM 108033	Cameroon: Southwest Province: Korup National Park
S. nux	SP 10628	CM 108034	Cameroon: Southwest Province: Korup National Park
S. nux	SP 10629	CM 108032	Cameroon: Southwest Province: Korup National Park
S. nux	SP 10571	CM 108035	Cameroon: Southwest Province: Baro
S. viridis	TM 37671	TM 37671	South Africa: Transvaal: Pafuri district
S. viridis	TM 37672	TM 37672	South Africa: Transvaal: Pafuri district
S. viridis	TM 37675	TM 37675	South Africa: Transvaal: Pafuri district
S. viridis	TM 37670	TM 37670	South Africa: Transvaal: Pafuri district
S. viridis	TM 37691	TM 37691	South Africa: Transvaal Province: Pafuri district
S. nigritellus	SP11041	CM 113647	Ghana: Greater Accra Region
S. nigritellus	SP11046	CM 113648	Ghana: Greater Accra Region
S. nigritellus	SP11047	CM 113649	Ghana: Greater Accra Region
S. nigritellus	SP11050	CM 113650	Ghana: Greater Accra Region
S. nigritellus	SP11111	CM 113644	Ghana: Greater Accra Region
S. nigritellus	SP11112	CM 113651	Ghana: Greater Accra Region
S. nigritellus	SP11113	CM 113652	Ghana: Greater Accra Region
S. nigritellus	SP10161	CM 113653	Ghana: Greater Accra Region

Table 1. continued

Species	Tissue Collection Number	Museum Catalog Number	Locality
S. viridis	TK 33264	CM 98039	Kenya: Coast Province: Kwale District
S. viridis	TK 33265		Kenya: Coast Province: Kwale District
S. viridis	TK 33266	CM 98040	Kenya: Coast Province: Kwale District
S. viridis	SP 5498	CM 102242	Kenya: Coast Province: Taita District
S. viridis	SP 5499	CM 102241	Kenya: Coast Province: Taita District
S. viridis	SP 5500	CM 102243	Kenya: Coast Province: Taita District
S. viridis	F 52119	uncataloged	South Africa
S. viridis	F 52120	uncataloged	South Africa
S. viridis	F52121	uncataloged	South Africa
S. viridis	TM 37673	TM 37673	South Africa: Transvaal: Pafuri district
S. viridis	TM 37689	TM 37689	South Africa: Transvaal: Pafuri district
S. viridis	TM 37674	TM 37674	South Africa: Transvaal: Pafuri district
S. viridis	TM 38144	TM 38144	South Africa: Transvaal: Pafuri district
S. viridis	TM 38142	TM 38142	South Africa: Transvaal: Pafuri district
S. viridis	TM 38143	TM 38143	South Africa: Transvaal: Pafuri district
S. viridis	TM 38149	TM 38149	South Africa
S. viridis	TM 39482	TM 39482	South Africa: Northern Province: Punda Milia
S. viridis	TM 39481	TM 39481	South Africa: Northern Province: Punda Milia
S. robustus	SMG 10560	FMNH 166186	Madagascar: Tsinjoarivo Forest
S. robustus	SMG 5930	FMNH 151939	Madagascar: Parc National de Zombitse-Vhibasia
S. sp nov..	RBJ 161	UADBA 46923	Madagascar: Province de Mahajanga
S. sp. nov.	RBJ 215	uncatologed	Madagascar
S. sp. nov.	SMG 14474	uncatologed	Madagascar
S. heathi	GenBank Accession AF376831	MVZ 176513	China: Yunnan Province
S. heathi	BRS 11	MVZ 176513	China: Yunnan Province
S. heathi	JLP 16924	MVZ 186416	Vietnam: Vinh Phu Province: Tam Dao
S. heathi		MVZ 186412	Vietnam: Vinh Phu Province: Tam Dao
S. heathi	F 42769	ROM 107786	Vietnam: Yok Don National Park
S. kuhlii	AK 21476	No voucher collected	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21477	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21478	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21479	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21480	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21481	no voucher	Malaysia: Kedah State: Jitra

Table 1. continued

Species	Tissue Collection Number	Museum Catalog Number	Locality
S. kuhlii	AK 21482	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21483	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21484	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21485	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21486	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21487	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21488	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21489	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21490	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21491	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21492	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21493	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	AK 21494	no voucher	Malaysia: Kedah State: Jitra
S. kuhlii	F 44160	ROM 110835	Vietnam: Cat Tien
S. kuhlii	F 44162	ROM 110837	Vietnam: Cat Tien
S. kuhlii	F 44165	ROM 110840	Vietnam: Cat Tien
S. kuhlii	F 44166	ROM 110841	Vietnam: Cat Tien
S. kuhlii	F 44167	ROM 110842	Vietnam: Cat Tien
S. kuhlii	F 44168	ROM 110843	Vietnam: Cat Tien
S. kuhlii	F 44282	ROM 110956	Vietnam: Soc Trang
S. kuhlii	F 44283	ROM 110957	Vietnam: Soc Trang
S. kuhlii	JLP 16928	MVZ 186418	Vietnam: Vinh Phu Province: Tam Dao
S. kuhlii	JLP 16936	MVZ 186421	Vietnam: Vinh Phu Province: Tam Dao
S. kuhlii	LRH 2945	uncataloged	Philippines
S. kuhlii	EAR 1266	uncataloged	Philippines
S. kuhlii	EAR 1371	uncataloged	Philippines
M. tricolor	SP 13200	CM 114030	Ethiopia: Shoa Province
E. serotinus	AK 10826	uncataloged	Azerbaijan:Gobustan
M. welwitschii	from GenBank	see Ruedi and Mayer, 2001	South Africa: Transvaal

The total numbers of specimens of each species are as follows: 56 *S. dinganii*, 20 *S. viridis*, 8 *S. nigritellus*, 9 *S. nux*, 2 *S. leucogaster*, 32 *S. kuhlii*, 4 *S. heathi*, 2 *S. robustus*, 3 *S. sp. nova*, and 1 *S. nigrita*. Outgroup taxa include *Myotis welwitschii* (GenBank Accession AF376874; Ruedi and Mayer, 2001) and *Eptesicus serotinus* (AK 10628) for the cytochrome *b* dataset, *Eptesicus serotinus* (AK 10628) and *Myotis tricolor* (CM 114030) for the Y chromosome data set, and *Eptesicus serotinus* (AK 10628) for the combined data set. There exists no well supported phylogeny for vespertilionines and as such, the outgroup taxa were chosen because the genus *Scotophilus* has been shown to be monophyletic (Hoofer and Van Den Bussche, 2003), and the outgroup taxa represent vespertilionines that share a closer relationship to *Scotophilus* than other chiropterans.

Laboratory Methods and Data Collection

Tissue samples used for genetic analysis included heart, kidney, and/or liver. Total genomic DNA was extracted by established protocols (Maniatis et al., 1982). An aliquot of the total extracted DNA was electrophoresed on a 1% agarose gel to confirm successful extraction as well as to provide a rough estimate of DNA concentration. Extracted DNA was stored in microcentrifuge tubes at 4˚ C.

An approximately 1500 bp segment of the mitochondrial DNA was amplified via the Polymerase Chain Reaction (PCR; Saiki et al., 1988) utilizing primers LGL 765F and LGL 766R (Table 2) that amplify the entire cytochrome *b* gene. To isolate the Y chromosome segment of interest, approximately 2200 bp of Y chromosome DNA was amplified with primers 33X5YF and LGL 331 (Fig.2, Table 2). Amplifications were

conducted on a GeneAmp® PCR System 2700 (Applied Biosystems, Foster City, CA) as follows: a hot start of 3 minutes at 95° C followed by 32 cycles of 95° C for 45 s of denaturing, 50° C for 30 s of annealing, and 70° C for 2.5 minutes of extension with a final extension of 70° C for 5 minutes. Amplification reactions were 50 µl in volume and consisted of the following: 0.1- 0.5 µg genomic DNA; 5 µl 10X PCR buffer (0.1 M Tris-HCl, pH 8.5, 0.025 M $MgCl_2$, 0.5 M KCl), 5 µl 8 mM dNTP mix (2 mM dATP, dTTP, dCTP, Dgtp, in 0.1 M Tris-HCl, pH 7.9), 1 µl of a 10 mM solution of each primer (LGL 765F and LGL 766R for cytochrome *b* and 33X5YF and LGL 331 for zfy), 2.5 Units Amplitaq® *Taq* polymerase (Applied Biosystems, Foster City, CA), and brought to a volume of 50 µl with deionized water. The amplicons were then visualized on a 1% agarose gel to confirm amplification of the correct segment and to assess concentration of amplicon. A Qiagen PCR purification kit (Qiagen Inc., Valencia, CA) was used to clean up the amplified fragments and to further prepare the templates for sequencing. Cycle sequencing utilized ABI Prism® BigDye™ Terminators v 2.0 and v 3.0 (Applied Biosystems, Foster City, CA) and was conducted on a GeneAmp® PCR System 2700 as follows: 25 cycles of 96° C for 30 s of denaturing, 50° C for 15 s of annealing, and 60° C for 4 minutes of extension. Sequencing primers for cytochrome *b* sequencing were LGL 765F, LGL 766R, and 388F (Table 2), an internal primer designed for completely sequencing through the entire cytochrome *b* gene. The complete (1,140 bp) cytochrome *b* gene was sequenced with these primers. Sequencing primers for *zfy* were 33X5YF, 33X6R, 33X6F, and LGL 331 (Table 2). Sequence reactions were then purified using

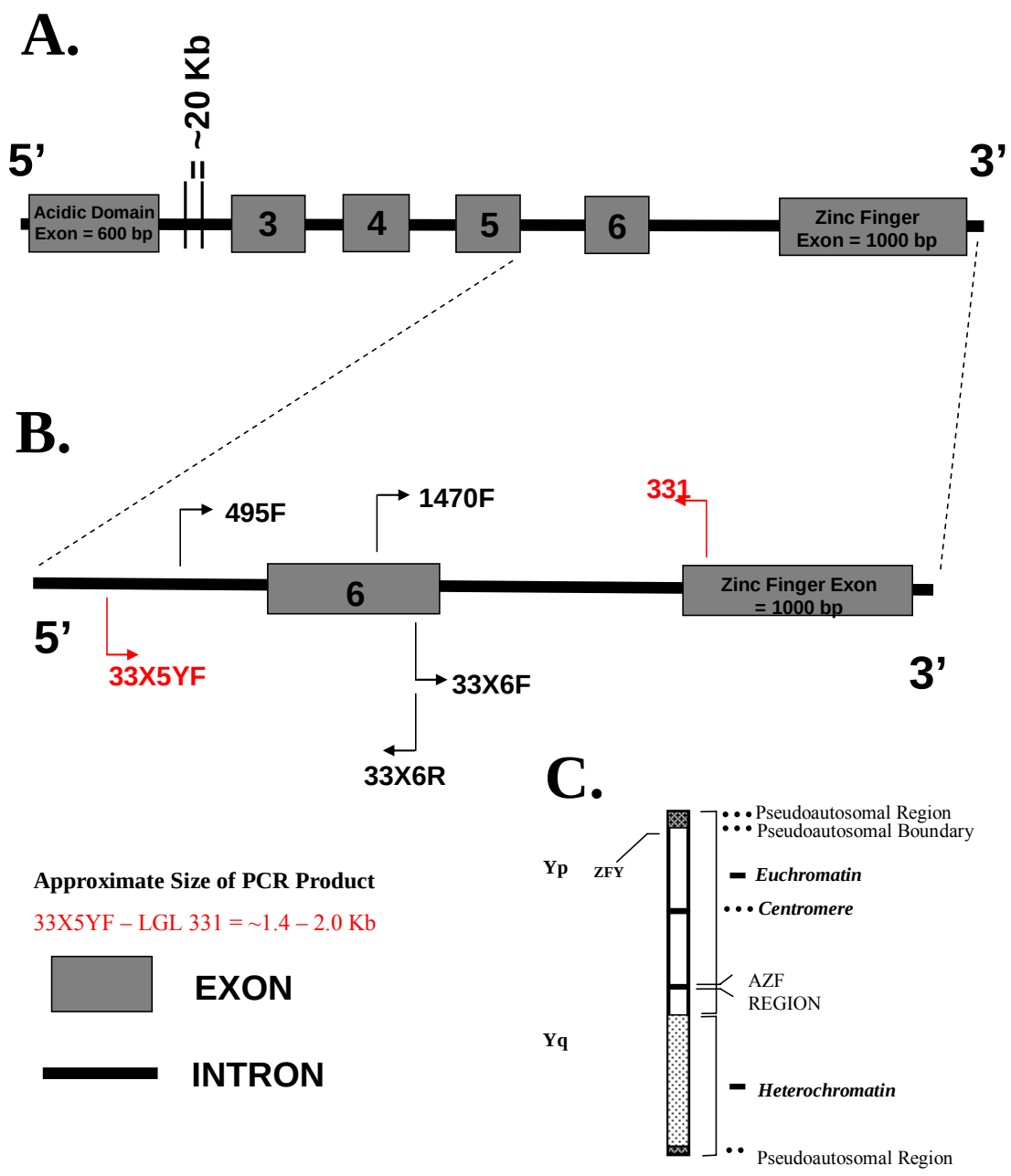


Figure 2. The *zfy* gene region. **A.** Genetic architecture of the *zfy* gene region. **B.** Primer placement on the segment of interest used in this study. **C.** The *zfy* gene is located on the p arm of the y chromosome below the pseudoautsomal boundary (redrawn from Human Chromosomes Fourth Edition, Figure 17.3, Miller and Therman, 2001).

sephadex spin columns. The purified sequence reactions were then dehydrated in a speedvac, and frozen until sequence visualization. Sequences were visualized and data was collected on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA). Raw sequences were automatically analyzed using Sequencing Analysis Software ™ (Applied Biosystems, Foster City, CA) and then saved to a disk for further analysis. Sequences were aligned by eye and ambiguities were called in Sequencher v 4.1 (Gene Codes Corporation, Ann Arbor, MI) for the cytochrome *b* sequences. The *zfy* sequences

Table 2. The three cytochrome *b* primers and six y chromosome primers used in this study. Primers LGL 765 F and LGL 766 R were used to amplify a region of the mitochondrial genome that contains the cytochrome b gene. These two primers along with Scot cyt b 388 F were also used as sequencing primers to sequence across the entire cytochrome b gene. Primers 33X5Y F and 331 were used to amplify a segment of the y chromosome. These two primers along with primers 33X6 F, 33X6 R, Scot *zfy* 495 F, and Scot *zfy* 1470 F were used to sequence a portion of two introns and all of exon 6 of the zinc finger y gene.

Primer Name	Primer Sequence	Primer Reference
LGL 765 F	5' - GAA AAA CCA YCG TTG TWA TTC AAC T - 3'	Bickham et al., 1995
LGL 766 R	5' - GTT TAA TTA GAA TYT YAG CTT TGG G - 3'	Bickham et al. 2004
388 F	5' - GGY TAT GTT CTY CCA TGA GG - 3'	This study
331	5' - GCA AAT CAT GCA AGG ATA GAC - 3'	unpublished
33X5Y F	5' - GCA GCA GCT TAT GGT AAG TGA - 3'	unpublished
33X6 F	5' - RGC AGT ACC AAA CAG GTG AGG - 3'	unpublished
33X6 R	5' - CCC TCA CCT GTT TGG TAY TGC - 3'	unpublished
Scot *zfy* 495 F	5' - TAG GTA CAT GGA CTT TCA GC - 3'	This study
Scot *zfy* 1470 F	5' - TTA GGT GAT AAT TCT GAC GG - 3'	This study

were submitted to CLUSTAL X (Thompson et al. 1997) for alignment utilizing the default gap-cost ratio (15.00:6.66) and again with a gap-cost ratio of 5:4. The use of two gap-cost ratios allowed for verification of the placement of indels in the alignment of all *zfy* sequences. The resultant alignments were refined by eye and ambiguities called in Sequencher v 4.1 (Gene Codes Corporation, Ann Arbor, MI).

Methods of Phylogenetic Inference

A neighbor joining tree (NJ) based on Kimura 2-parameter (K2P) distances was constructed for both the cytochrome *b* and the *zfy* datasets to allow for the determination of unique haplotypes in each of the two datasets. All subsequent phylogenetic analysis for both datasets involved data matrices composed of a single representative of each respective haplotype for each of the two datasets.

A maximum parsimony (MP) analysis was performed on each dataset (cytochrome *b* and Y chromosome) and on a combined data matrix (if warranted based on an incongruence length difference test; Farris et al., 1994). A branch-and-bound option was used for the Y chromosome dataset and the heuristic search option for the cytochrome *b* dataset with tree-bisection-and-reconnection (TBR) branch swapping in PAUP version 4.0b4a (Swofford, 1999). Starting trees were obtained via stepwise addition. Data were polarized via the outgroup method, and the outgroup taxa were chosen based on the study of Hoofer and Van Den Busche (2003). Phylogenetically informative characters were unordered and equally weighted with gaps treated as missing data. Stability of clades was examined by bootstrap analysis (Felsenstein, 1985). Bootstrap analysis for both datasets consisted of 1,000 pseudoreplicates with

resampling of all characters, heuristic searching, and tree-bisection-and-reconnection (TBR) branch swapping.

A maximum likelihood (ML) analysis of each data set and the combined data matrix (if warranted) was performed in PAUP version 4.0b4a (Swofford, 1999). The best fit ML model for each data set was determined using MODELTEST Version 3.06 (Posada and Crandall, 1998). MODELTEST helps choose the model of DNA substitution that best fits the data set through hierarchical hypothesis testing with the use of likelihood ratio tests and the Akaike information criterion (AIC) (Akaike, 1974). MODELTEST tests 56 nested models based on eight popular models of DNA evolution plus transition and transversion rates, rates at different sites, and the proportion of invariant sites. The eight popular models incorporated into MODELTEST are: Jukes and Cantor (Jukes and Cantor, 1969), F81 (Felsenstein, 1981), HKY 85 (Hasegawa et al., 1985), Tamura and Nei (Tamura and Nei, 1993), Kimura 2-parameter (Kimura, 1980), Kimura 3-parameter (Kimura 1981), SYM (Zharkikh, 1994), and GTR (Rodriguez et al., 1990). After model evaluation and selection of the best fit model, the maximum likelihood tree for each data set and for the combined data set was constructed using a heuristic search. Bootstrap analysis for both datasets consisted of 100 pseudoreplicates with resampling of all characters, fast-heuristic searching, and tree-bisection-and-reconnection (TBR) branch swapping.

A Bayesian analysis was also performed using MrBayes (Huelsenbeck and Ronquist, 2001). This program utilizes Markov Chain Monte Carlo (MCMC) methods for the Bayesian inference of phylogeny and is based on the posterior probability of a

phylogenetic tree given an observed aligned matrix of DNA sequence data. The Bayesian analysis was implemented for 1 X 10^6 generations with one cold and three incrementally heated Markov chains, random starting trees for each chain, trees sampled every ten generations, and the analysis repeated three independent times to insure convergence of the chains to the same posterior probability distribution and that the likelihoods reached stable values (Huelsenbeck et al., 2002) for each dataset. Values for model parameters were not defined *a priori* in the analysis (from MODELTEST) but were treated as unknown variables with uniform priors in each Bayesian analysis (Leaché and Reeder, 2002).

RESULTS

Cytochrome *b*

A total of 1,140 bp were sequenced for 138 individuals (including two outgroup taxa) of which 507 bp were variable. Cytochrome *b* sequence data was also retrieved from GenBank for two individuals (*S. heathi* (MVZ 176513, GenBank accession AF376831) and *M. welwitchii* (GenBank accession AF376874); Ruedi and Mayer, 2001). In addition to the GenBank sequence for MVZ 176513, I also sequenced MVZ 176513 and my sequence differed from the published GenBank sequence by 10 base pairs (see appendix 1 for more details). Of the 507 variable bp, 431 bp were parsimony informative. A neighbor joining tree constructed in PAUP allowed me to identify 88 unique cytochrome *b* haplotypes (Table 3). An analysis of these 88 haplotypes based on maximum parsimony resulted in 768 equally parsimonious trees of 1554 steps. A strict consensus tree of the 768 equally parsimonious trees is shown in figure 3. A successive approximations approach (Farris, 1969) resulted in three equally parsimonious trees and a strict cocnsensus tree (not shown) of these three trees is identical to figure 3.

Figure 3. Strict consensus tree of 768 equally parsimonious cladograms of 88 cytochrome *b* haplotypes. The phylogeny was inferred using Maximum Parsimony optimaltiy criteria in PAUP. Geographic localities of the 15 major clades are as follows: 1 = Vietnam, Malaysia, Philippines; 2 = Kenya, Cameroon; 3 = Ghana; 4 = Madagascar; 5 = Vietnam, China; 6 = Madagascar; 7 = South Africa; 8 = Ghana; 9 = South Africa; 10 = Kenya; 11 = Ghana; 12 = Kenya; 13 = Ethiopia; 14 = Kenya; and 15 = Ethiopia, Kenya. Clade 13 includes a haplotype representing *S. nigrita* from the country of Kenya. The individual specimens in each clade are listed in Appendix B. The outgroup taxa are *Myotis welwitschii* and *Eptesicus serotinus*.

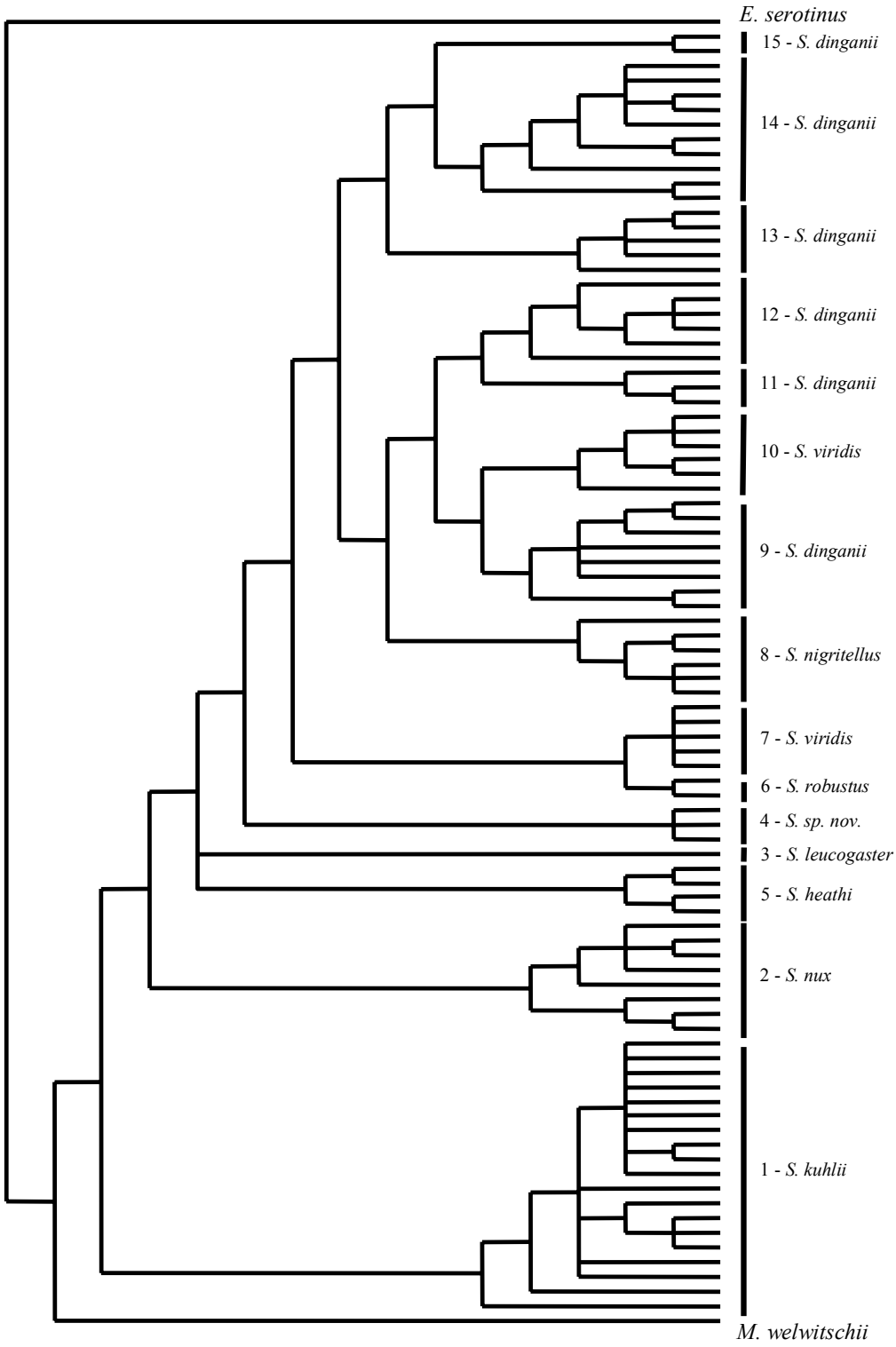

E. serotinus
15 - S. dinganii
14 - S. dinganii
13 - S. dinganii
12 - S. dinganii
11 - S. dinganii
10 - S. viridis
9 - S. dinganii
8 - S. nigritellus
7 - S. viridis
6 - S. robustus
4 - S. sp. nov.
3 - S. leucogaster
5 - S. heathi
2 - S. nux
1 - S. kuhlii
M. welwitschii

Table 3. The 88 cytochrome *b* haplotypes identified in this study.

Haplotype	Taxa	Haplotype	Taxa	Haplotype	Taxa
1	AK 21163	32	TK 33265	57	SP 5055
2	SP 5368	33	SP 7731	58	TK 33485
3	TK 33360	34	SP 7789	59	SP 5054
	TK 33361	35	TM 39625	60	SP 10620
	TK 33359		TM 39624	61	SP 10629
4	TK 33395	36	TM 37669	62	SP 10628
5	SP 5451		TM 37655	63	RBJ 161
	SP 5386		TM 38151	64	RBJ 215
6	SP 5370	37	TM 37668	65	SMG 14474
7	SP 5369		TM 37656	66	MVZ 176513 gb
8	TK 33141	38	F 52131	67	MVZ 176513
	SP 5083	39	SP 7732	68	ROM 107786
9	TK 33149	40	SP 7755	69	MVZ 186412
	TK 33142	41	SP 11111		MVZ 186416
10	TK 33189	42	SP 11112	70	AK 21494
11	SP 5454	43	SP 11050		AK 21476
12	SP 5452	44	SP 11113		AK 21478
	SP 5453	45	SP 10161		AK 21483
	SP 5385		SP 11046		AK 21487
13	AK 21213		SP 11047		AK 21488
	AK 21214	46	SP 11041		AK 21490
	AK 21215	47	TM 37691	71	AK 21479
	AK 21234		TM 37673		AK 21493
	AK 21235		TM 37674	72	AK 21485
14	AK 21223		TM 37689	73	AK 21481
15	AK 21259		TM 38142	74	ROM 110957
16	SP 5505		TM 38143	75	ROM 110837
17	SP 13027	48	TM 38144	76	ROM 110842
18	SP 5051		F 52119	77	ROM 110956
19	SP 5052		F 52120	78	AK 21482
20	SP 5078		F 52121	79	AK 21489
21	SP 5077		TM 37670	80	AK 21477
	TK 33535		TM 37671		AK 21480
	TK 33140		TM 37672		AK 21486
	SP 5084	49	TM 38149		AK 21491
22	SP 5082	50	TM 37675	81	AK 21484
	TK 33536	51	TM 39482		AK 21492
23	TK 33534		TM 39481	82	ROM 110835
24	SP 10181	52	FMNH 151939	83	ROM 110841
25	SP 10179	53	FMNH 166186	84	ROM 110843
26	SP 10180	54	SP 10136	85	ROM 110840
27	SP 5498		SP 10137	86	MVZ 186421
28	SP 5500	55	SP 10571	87	MVZ 186418
29	SP 5499	56	SP 5053	88	EAR 1266
30	TK 33266		SP 5056		LRH 2945
31	TK 33264		TK 33519		EAR 1371

A bootstrap consensus tree was calculated based on 1000 pseudoreplicates with re-sampling of 1,140 characters (Fig. 4). The phylogeny produced via MP has 15 well supported clades (Fig. 4). First, a clade comprising the entire genus is highly supported with bootstrap support of 100 (Node A). A clade composed of *S. kuhlii* (Clade 1) from three Southeast Asian localities (Philippines, Malaysia, and Vietnam) is well supported and is basal to the rest of *Scotophilus* (Node B). An unresolved area of the tree involves a polytomy of four Scotophilus species and one major clade containing multiple *Scotophilus* species. There is a strongly supported group of 5 haplotypes representing eight *S. nux* (Clade 2), a strongly supported group of 4 haplotypes representing three *S. heathi* (Clade 5), a single haplotype representing two identical individuals of *S. leucogaster* (Clade 3), and 3 haplotypes representing a new species of *Scotophilus* from Madagascar (Clade 4).

Within the largely African clade (Node C), high bootstrap values supporting various species level clades are present. A cluster of seven haplotypes is the basal group to this larger clade. It includes five haplotypes representing *S. viridis* (Clade 7) that clusters together with a cluster of two haplotypes representing *S. robustus* from Madagascar (Clade 6). The next clade (Node D) in this major cluster is largely composed of *S. dinganii* but includes one group of *S. viridis* (Clade 10) and one group of *S. nigritellus* (Clade 8) within this clade. This clade is further subdivided into two clades (Nodes E and F). The first clade (Node E) is composed of two major groups. One group includes four Ethiopian *S. dinganii* haplotypes and a single *S. nigrita* haplotype (Clade 13) and another group is composed of two very divergent *S. dinganii* haplotypes from

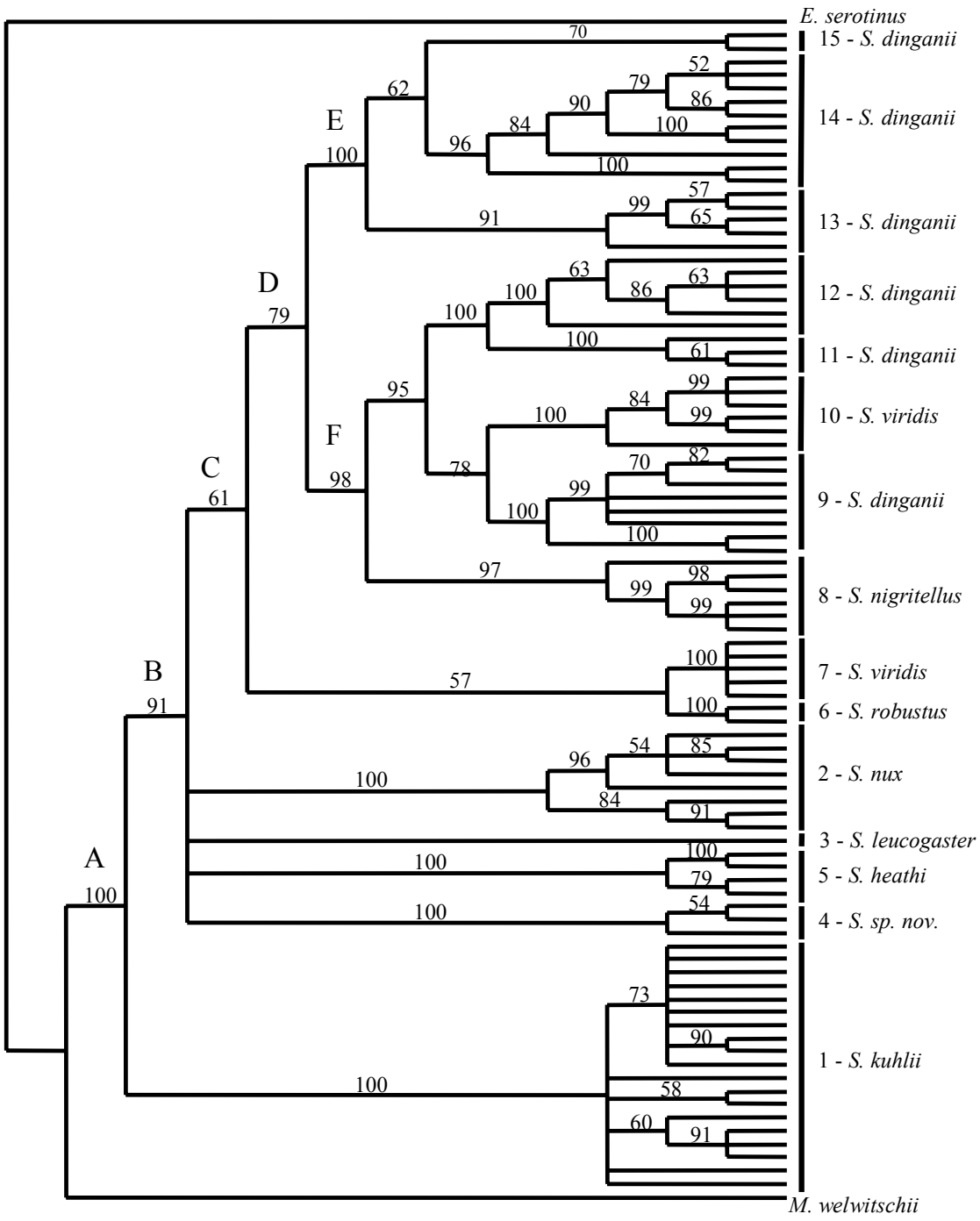


Figure 4. Maximum parsimony bootstrap consensus cladogram of 88 cytochrome *b* haplotypes. Geographic localities of the 15 major clades are as in figure 3. Clade 13 includes a haplotype representing *S. nigrita* from the country of Kenya. The outgroup taxa are *Myotis welwitschii* and *Eptesicus serotinus*.

southwestern Ethiopia and northwestern Kenya (Clade 15) that share a sister group relationship with 10 haplotypes representing *S. dinganii* from eastern Kenya (Clade 14). The second clade (Node F) is composed of four major groups. In this clade *S. dinganii* from the forests of western Kenya, (Clade 12), share a sister group relation with *S. dinganii* from coastal Ghana (Clade 11). The sister group to clade 12 and 11 is composed of two clades and includes a group of *S. viridis* (Clade 10) from eastern Kenya and a group of *S. dinganii* from northwestern South Africa (Clade 9).

A maximum likelihood (ML) analysis of the entire cytochrome *b* gene was accomplished in PAUP. The program MODELTEST selected the GTR + Γ + I model as the best fit model of nucleotide substitution. Nucleotide frequencies were set to A = 0.3247, C = 0.2798, G = 0.1080, and T = 0.28750. The gamma shape parameter was set to $\alpha = 1.4748$ and the assumed proportion of invariable sites was set to 0.4861. The ML analysis resulted in a maximum likelihood tree with a score of –Ln likelihood = 8687.99 (Fig. 5) that is identical to the strict consensus tree based on MP (Fig. 3) except for the relationship between tip clades 4 and 5. A bootstrap consensus tree shows the lack of support for several nodes present in the ML tree (Fig. 6). Again we see 15 clades present in these trees corresponding to the same clades seen in the phylogeny inferred through MP. The ML tree shows *S. kuhlii* as the basal *Scotophilus* (Clade 15). The topology of the ML bootstrap consensus tree (Fig. 6) is identical to the topology of the MP bootstrap consensus tree (Fig. 4) except for the collapse of node D in the ML bootstrap consensus tree. The bootstrap consensus tree (Fig. 6) shows node D collapsed

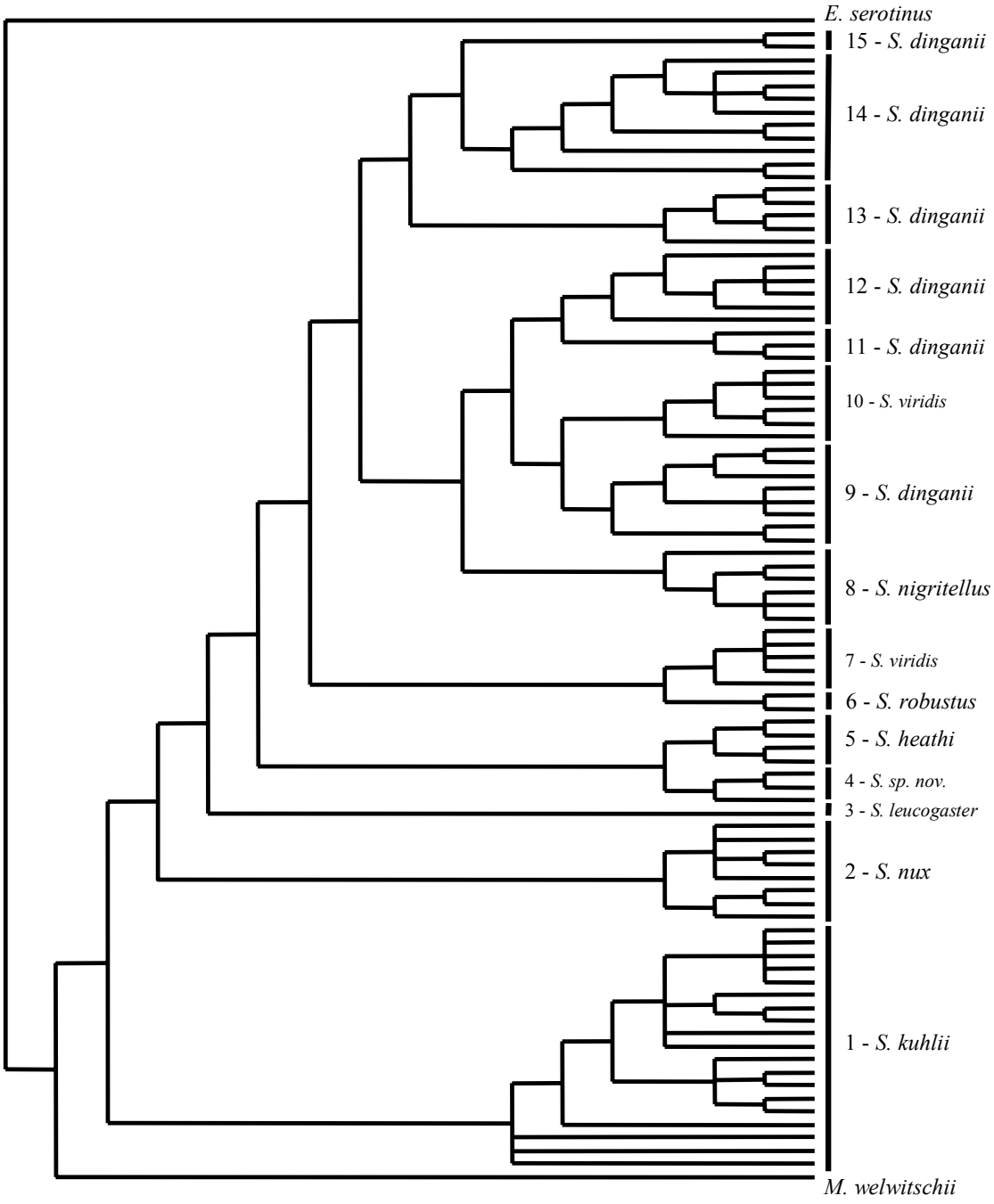


Figure 5. Maximum likelihood (-Ln = 8660.84) cladogram of 88 cytochrome *b* haplotypes. The phylogeny was constructed in PAUP using the GTR + Γ + I model of nucleotide substitution. Geographic localities of the 15 major clades are as in Figure 3. Clade 13 includes a haplotype representing *S. nigrita* from the country of Kenya. The outgroup taxa are *Myotis welwitschii* and *Eptesicus serotinus*.

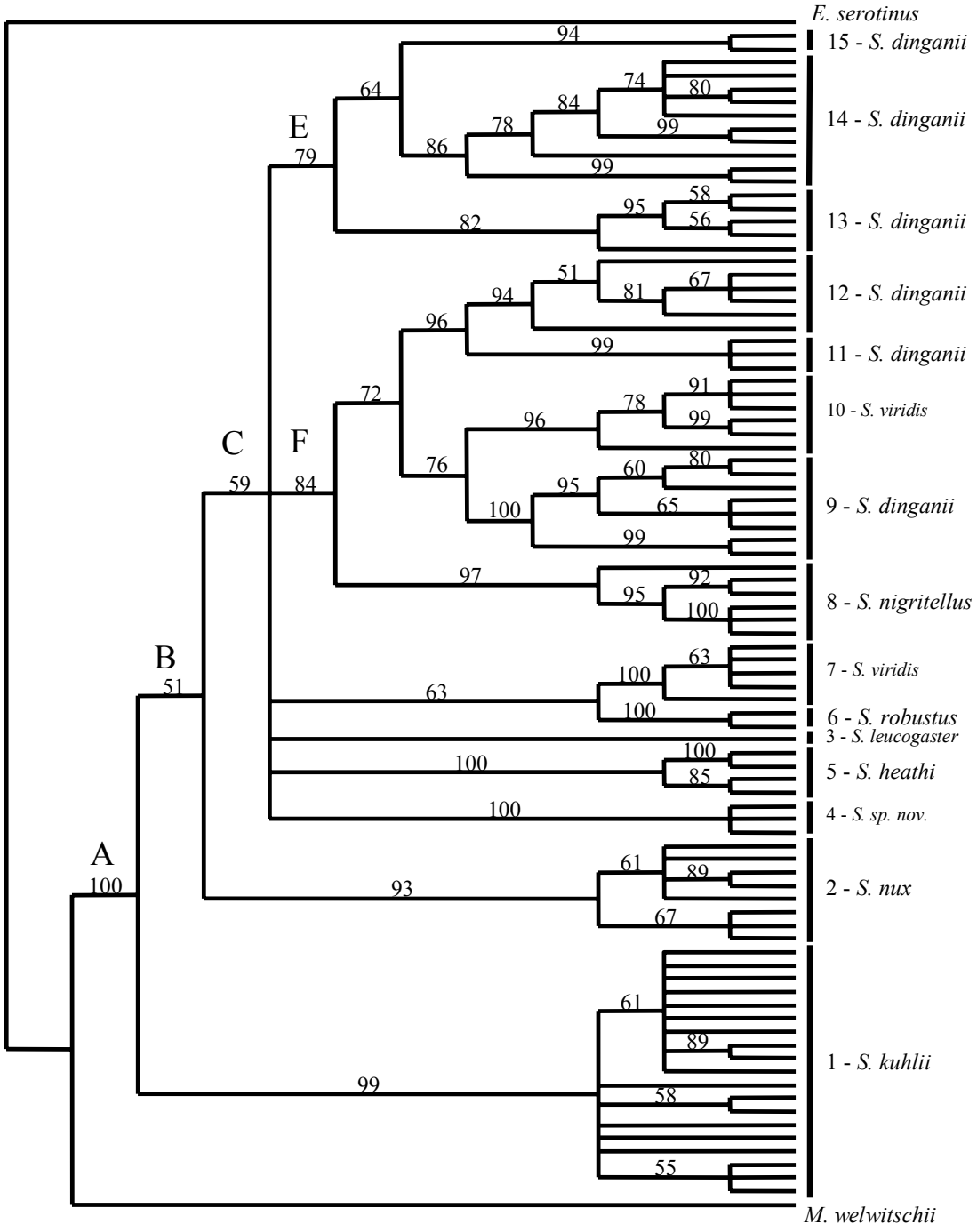


Figure 6. Maximum likelihood bootstrap consensus cladogram of 88 cytochrome *b* haplotypes. The phylogeny was constructed in PAUP using the GTR + Γ + I model of nucleotide substitution. Geographic localities of the 15 major clades are as in Figure 3. Clade 13 includes a haplotype representing *S. nigrita* from the country of Kenya. The outgroup taxa are *Myotis welwitschii* and *Eptesicus serotinus*.

(bootstrap support is less than 50%). Bootstrap support values for the 15 clades are all above 70%.

The Bayesian analysis of the cytochrome *b* data under the GTR + Γ + I model of nucleotide substitution produced a phylogeny (Fig. 7) similar to the ML phylogeny (Fig. 5) except that the Bayesian analysis shows more resolution than either the MP or ML bootstrap consensus trees, and the Bayesian tree shows weak support for a (*S. heathi* + *S. sp. nov.* Madagascar) sister group relationship (0.56 posterior probability). The three independent analyses converged on stable posterior probability values after a burn in time of 100,000 generations. The nucleotide substitution model parameter estimates (GTR + Γ + I) are given in table 4. The phylogeny inferred has a log likelihood score of –Ln = 8758.16 and shows high posterior probability support for the same 15 clades as recovered in the MP and ML analysis.

Table 4. Nucleotide substitution parameter estimates under the GTR + Γ + I model from a Bayesian analysis of the cytochrome *b* data.

	Mean	Variance	95%CI		Median
TL	3.409948	0.081379	2.960000	4.043000	3.367000
r_{GT}	1.000000	0.000000	1.000000	1.000000	1.000000
r_{CT}	26.058693	157.765389	10.810885	58.357558	23.187539
r_{CG}	1.974520	1.435610	0.534287	4.986109	1.658324
r_{AT}	1.001530	0.298936	0.347970	2.303000	0.850840
r_{AG}	34.250170	275.188063	14.310743	74.395722	30.981778
r_{AC}	1.847491	0.938545	0.703116	4.246191	1.605514
π_A	0.334169	0.000137	0.311288	0.356757	0.333861
π_C	0.289653	0.000093	0.272127	0.309822	0.289524
π_G	0.089126	0.000041	0.076926	0.101786	0.089066
π_T	0.287052	0.000101	0.267820	0.307225	0.286835
α	1.598472	0.063005	1.156574	2.145733	1.577146
Pinvar.	0.450002	0.000321	0.414093	0.483970	0.450460

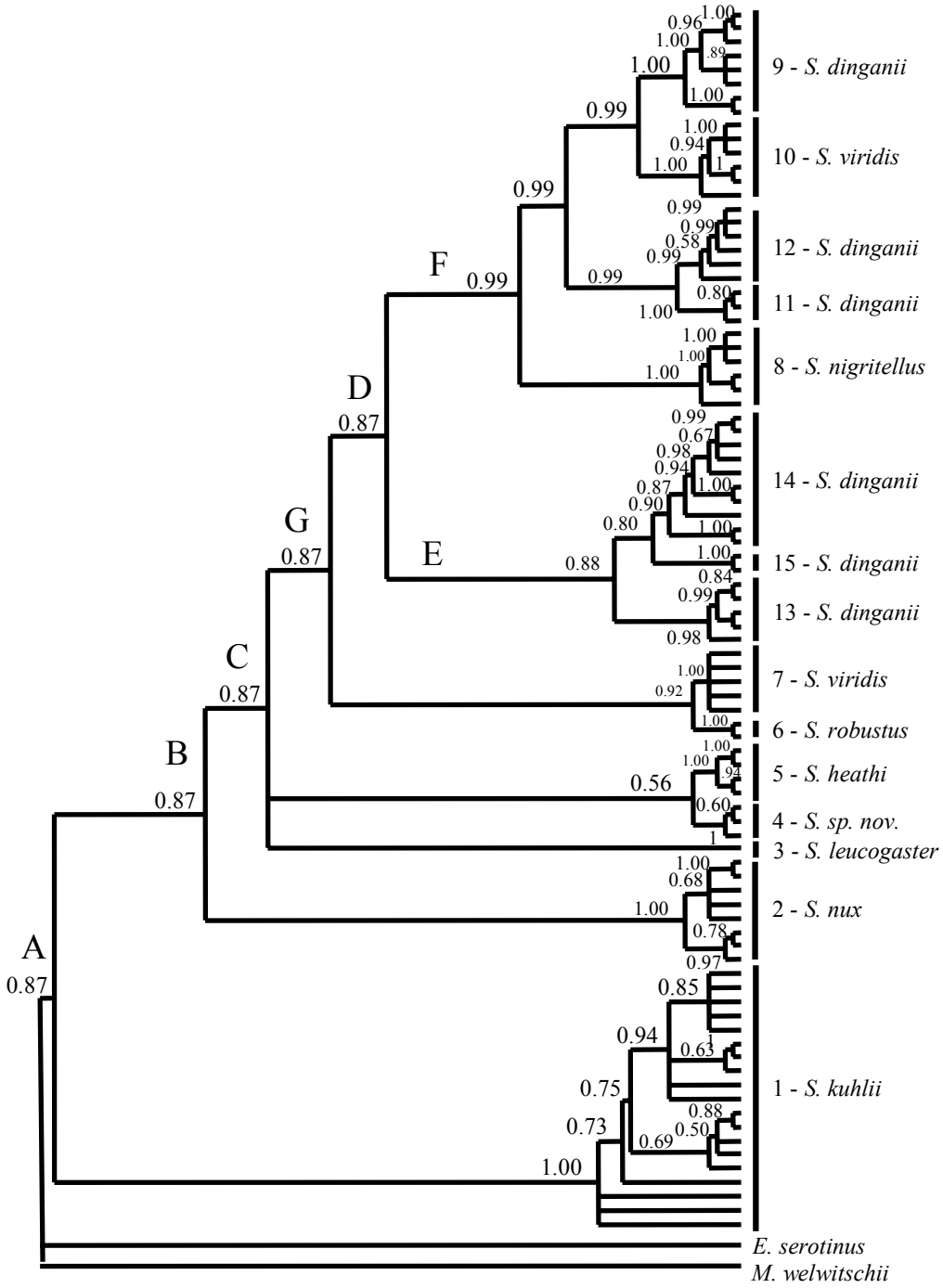


Figure 7. The 50% majority rule consensus tree from a Bayesian analysis of 88 cytochrome *b* haplotypes. Numbers on nodes represent posterior probability values. Geographic localities of the 15 major tip clades are as in figure 3. Clade 13 includes a haplotype representing *S. nigrita* from the country of Kenya. The outgroup taxa are *Myotis welwitschii* and *Eptesicus serotinus*.

Table 5. Pairwise genetic distances (Kimura 2- parameter) between the 15 clades in the cytochrome *b* phylogeny for *Scotophilus*. The locality codes for Taxa with more than one entry are as follows: S.A. = South Africa, K = Kenya, G = Ghana, and E = Ethiopia. The Kimura 2-parameter distances are below the diaganol and the actual numbers of nucleotide changes are above the diaganol.

Clade	Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	outs
1	*S. kuhlii*	—	174	167	165	172	180	162	169	177	170	173	168	172	181	169	236
2	*S. nux*	0.18	—	146	149	168	156	151	148	147	142	151	150	146	147	146	248
3	*S. leucogaster*	0.17	0.15	—	130	143	134	135	129	123	126	135	130	140	137	125	242
4	*S. sp. nov.*	0.17	0.15	0.13	—	136	127	127	128	127	130	129	126	123	133	132	245
5	*S. heathi*	0.18	0.17	0.14	0.13	—	144	161	159	147	145	153	150	160	159	159	248
6	*S. robustus*	0.20	0.17	0.13	0.13	0.15	—	105	113	112	119	115	118	124	125	125	245
7	*S. viridis*	0.16	0.15	0.14	0.13	0.16	0.10	—	105	112	115	118	112	113	115	114	246
8	*S. nigritellus*	0.17	0.15	0.13	0.12	0.16	0.11	0.10	—	59	65	73	65	93	91	87	256
9	*S. dinganii* S.A.	0.18	0.14	0.12	0.13	0.15	0.12	0.11	0.05	—	46	63	57	99	101	96	253
10	*S. viridis* K	0.18	0.14	0.12	0.13	0.15	0.12	0.11	0.06	0.04	—	63	56	102	106	101	246
11	*S. dinganii* G	0.17	0.15	0.13	0.12	0.15	0.11	0.11	0.06	0.05	0.05	—	35	106	106	105	248
12	*S. dinganii* K	0.18	0.15	0.13	0.12	0.15	0.12	0.11	0.06	0.05	0.05	0.03	—	98	102	99	242
13	*S. dinganii* E	0.18	0.14	0.14	0.12	0.16	0.12	0.11	0.09	0.09	0.10	0.10	0.09	—	59	49	244
14	*S. dinganii* K	0.18	0.15	0.13	0.12	0.15	0.12	0.11	0.09	0.09	0.10	0.09	0.10	0.05	—	42	247
15	*S. dinganii* E	0.17	0.14	0.12	0.13	0.16	0.12	0.11	0.08	0.09	0.10	0.10	0.09	0.05	0.03	—	248
	outgroups	0.25	0.26	0.25	0.25	0.26	0.25	0.25	0.27	0.26	0.26	0.25	0.25	0.26	0.25	0.26	—

Pairwise genetic distance measures (Kimura 2 parameter distances) are presented in Table 5 for the 15 major clades presented and the outgoup taxa based on cytochrome *b* data. Genetic distances ranged from 3% to 20% within *Scotophilus* and from 25% to 27% between *Scotophilus* and the outgroup taxa. The absolute numbers of differences are also presented in table 5. They range from 35 to 181 changes within *Scotophilus* and from 236 to 248 changes between *Scotophilus* and the two outgroup taxa.

zfy Data

A total of 51 individuals were sequenced including two outgroup taxa. The total number of base pairs sequenced per individual ranged from 1,597 bp to 2,100 bp and averaged 1,963 bp per individual. A total of 2,283 total characters were included in the *zfy* data matrix (including two outgroup taxa). This includes 12 insertion deletion events that account for 276 characters in the data matrix (Table 6). Of these 2,283 characters,

Table 6. Insertion/deletion events present in a portion of the *zfy* region in *Scotophilus*. The size is given in base pairs (bp) and the position refers to the position on the aligned data matrix.

data matrix position	size (bp)	description
111 - 127	17	deletion in *S. kuhlii*
186 - 337	152	insertion in *S. lecogaster*
507 - 508	2	deletion in *S. viridis*
553	1	deletion in *S. kuhlii*
566-568	3	insertion in *S. kuhlii* and *S. nux*
769 - 772	4	deletion in *S. kuhlii*
920 - 970	50	deletion in *S. nux*
993 - 999	7	deletion in *S. kuhlii*
1008 - 1014	7	insertion in *S. kuhlii*
1092	1	insertion in *S. kuhlii*
1201 - 1231	31	insertion in *S. kuhlii* and *S. nux*
1248	1	insertion in *S. kuhlii*

1,997 characters were constant and 132 characters were parsimony informative. Excluding the outgroup taxa, 93 total characters were variable with 76 of these characters being parsimony informative. Construction of a neighbor joining tree allowed me to identify 20 unique *zfy* haplotypes (Table 7). An analysis of these 20 haplotypes based on maximum parsimony resulted in 2 equally parsimonious trees of 315 steps in length. Both of the equally parsimonious trees had the following description: consistency index = 0.9651, homoplasy index = 0.0349, rescaled consistency index = 0.9239, and a retention index of 0.9574.

Table 7. The 20 *zfy* haplotypes identified in this study.

Haplotype	Taxa	Haplotype	Taxa	Haplotype	Taxa
1	AK 21163	8	SP 5084	15	SP 10137
	AK 21235		SP 5082	16	SP 10136
	TK 33149	9	SP 5077		ROM 110843
	SP 5370	10	SP 5051	17	MVZ 186421
	SP 5386		TM 37656		ROM 110840
2	TM 37672	11	TM 37655	18	ROM 110841
	TM 37671		TM 37668		AK 21491
	TM 38149		TM 37669		AK 21489
	TM 37675		SP 7789		AK 21494
	TM 38143		SP 7755		AK 21488
3	AK 21223		SP 7732		MVZ 186418
4	SP 10179		SP 7731		AK 21493
5	SP 10181	12	MVZ 186412	19	AK 21490
6	SP 10180	13	MVZ 176513	20	SP 10628
	SP 11111		MVZ 186416		SP 10620
7	SMG 14474	14	SP 5505		SP 5056
					SP 5053

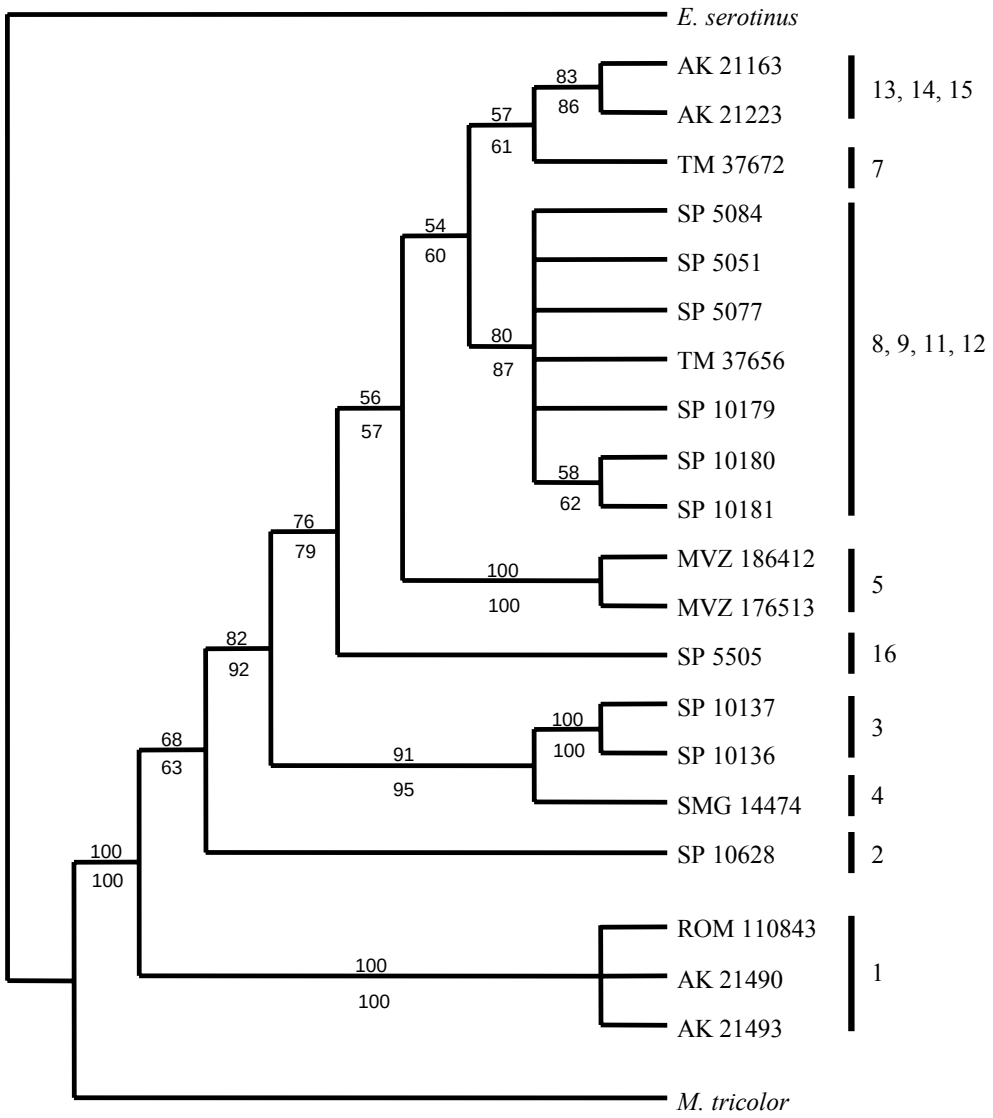


Figure 8. Maximum likelihood and maximum parsimony cladograms for the *zfy* data. The maximum likelihood cladogram (-Ln likelihood = 4745.99) is based on the HKY + Γ model of nucleotide substitution and the maximum parsimony cladogram (tree score =315) from an analysis of 20 *zfy* haplotypes representing eight species of *Scotophilus*. The numbers above the nodes are bootstrap support values for the maximum likelihood tree and those below are bootstrap support values for the maximum parsimony tree. The clades are numbered as in the cytochrome *b* phylogenies and are as follows: 1= *S. kuhlii* from Vietnam and Malaysia; 2 = *S. nux* from Cameroon and Kenya; 3 = *S. leucogaster* from Ghana; 4 = *S. sp.* from Madagascar; 5 = *S. heathi* from China and Vietnam; 7 = *S. viridis* from South Africa; 8,9,11,12 = *S. dinganii* from Kenya, Ghana, and South Africa; 13, 14, 15 = *S. dinganii* from Ethiopia; and 16 = *S. nigrita* from Kenya. The outgroup taxa are *Myotis tricolor* and *Eptesicus serotinus*.

A bootstrap consensus tree was calculated based on 1000 pseudoreplicates with re-sampling of 2,283 characters (Fig. 8). The phylogeny shows *S. kuhlii* as the basal taxa with monophyly of the African *Scotophilus* supported. The basal African *Scotophilus* is *S. nux*. All *S. diganii* formed a monophyletic clade. *S. heathi* is embedded within the African clade forming a sister group relationship with *S. dinganii*, and thus monophyly of the Asian taxa is not supported. Also notable, *S. nigrita* is sister to the larger clade containing *S. heathi*, *S. dinganii*, and *S. nigritellus*; *S. nigrita* shows a more distant relationship with Ethiopian *S. dinganii* than that seen with cytochrome *b* data.

A maximum likelihood (ML) analysis of the *zfy* data set was accomplished in PAUP. The program MODELTEST selected the HKY + Γ model as the best fit model of nucleotide substitution. Nucleotide frequencies were set to A = 0.28890, C = 0.18020, G = 0.19920, and T = 0.33170. The gamma shape parameter was set to α = 1.1055 and the transition/transversion ratio was set to TRatio = 1.7937. The ML analysis resulted in a maximum likelihood tree with a score of –Ln likelihood = 4745.99 (Fig. 8). The ML tree has a topology identical to that of the MP analysis.

Table 8. Nucleotide substitution parameter estimates under the HKY + Γ model from a Bayesian analysis of the *zfy* data.

Parameter	Mean	Variance	95%CI		Median
TL	0.301593	0.000403	0.265000	0.344000	0.301000
K	3.819335	0.224068	2.967941	4.834668	3.789891
π_A	0.288364	0.000080	0.271274	0.306340	0.288313
π_C	0.180459	0.000058	0.166051	0.196306	0.180225
π_G	0.199773	0.000062	0.184209	0.215048	0.199795
π_T	0.331403	0.000089	0.312702	0.349759	0.331352
α	9.286013	152.847620	0.698079	44.405401	2.857486

The Bayesian analysis of the *zfy* data under the HKY + Γ model of nucleotide substitution produced a phylogeny (Fig. 9) identical to the ML and MP phylogenies (Fig. 8) with strong posterior probability support. The three independent analyses converged on stable posterior probability values after a burn in time of 30,000 generations. The model parameter estimates (HKY + Γ) are given in table 8. The phylogeny inferred has a log likelihood score of –Ln = 4768.58.

Pairwise genetic distance measures (Kimura 2 parameter distances) are presented in table 9 for the 20 haplotypes presented and the outgoup taxa based on the *zfy* data. Genetic distances ranged from 0.1% to 2.2% within *Scotophilus* and from 12.4% to 20.7% between *Scotophilus* and the outgroup taxa. The absolute number of nucleotide changes are also presented in table 9 and range from 1 to 41 changes within *Scotophilus* and from 115 to 178 changes between *Scotophilus* and the outgroup taxa.

Combined Data Matrix

Results of an Incongruence Length Difference (ILD, Farris et al., 1994) test as implemented in PAUP as the partition homogeneity test resulted in a test statistic of p = 0.24 thus failing to refute the null hypothesis that the two data sets are congruent. The cytochrome *b* and the *zfy* data set were then combined and a similar phylogenetic analysis based on the three optimality criteria was conducted. The total data matrix included 3,364 total characters (including all indels) of which 2,712 characters were constant, 190 characters were uninformative, and 462 informative characters. There were 42 haplotypes identified in the combined data set representing eight species of *Scotophilus*.

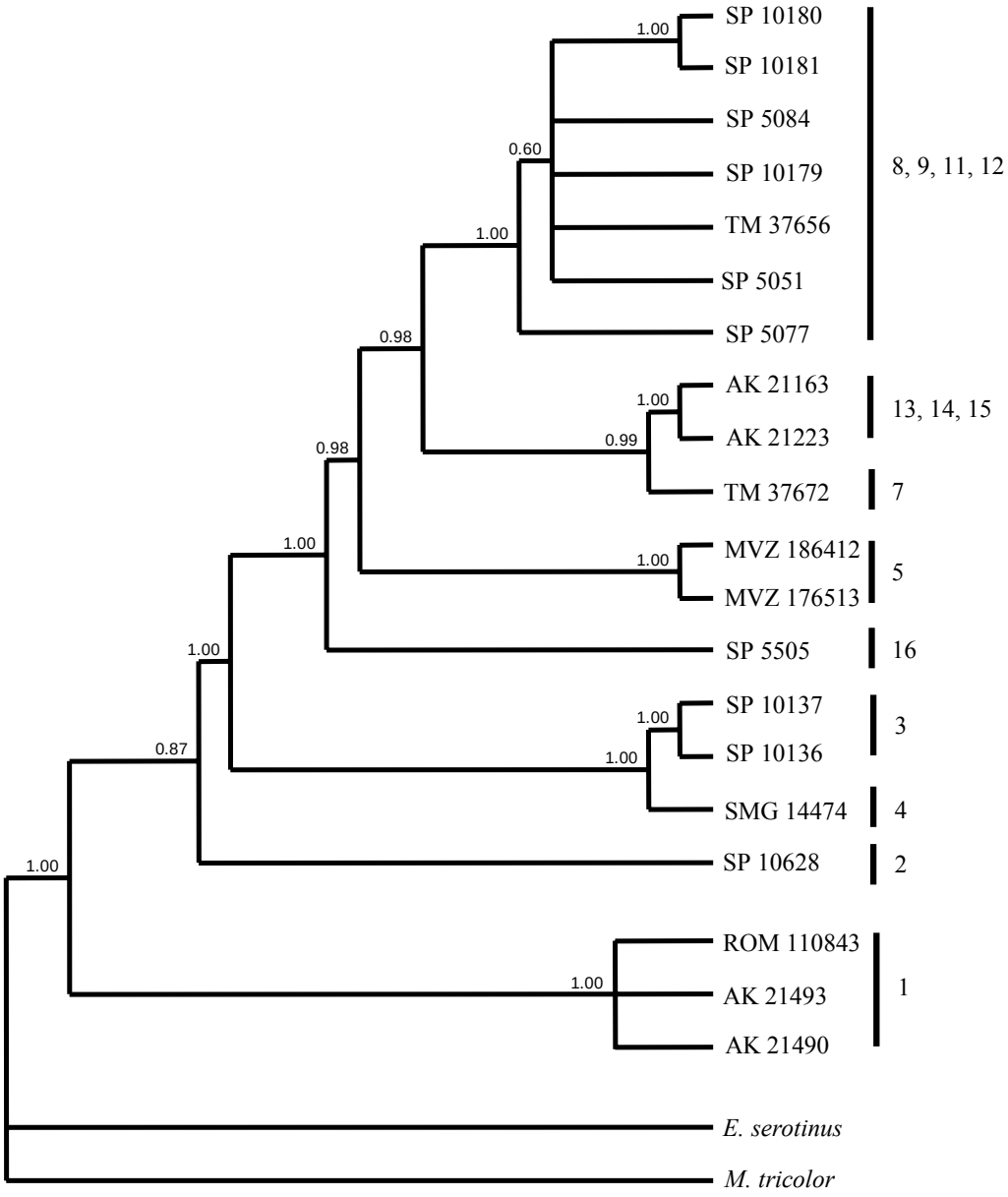


Figure 9. The 50% majority rule bootstrap consensus tree from a Bayesian analysis of 20 *zfy* haplotypes. The tree was computed based on the HKY + Γ model of nucleotide substitution. The numbers above the nodes are posterior probability values. The clades are labeled as in figure 8. The outgroup taxa are *Myotis tricolor* and *Eptesicus serotinus*.

Table 9. Pairwise genetic distances (Kimura 2- parameter below diaganol, absolute # differences above) between the 21 *zfy* haplotypes for *Scotophilus* and the two outgroup taxa. The locality codes for Taxa with more than one entry are as follows: V = Vietnam, M = Malaysia, C = Cameroon, K = Kenya, G = Ghana, and E = Ethiopia.

	1	2	3	4	5	6	7	8	9	10	11
E. serotinus	—	120	120	121	122	121	121	121	121	121	123
AK 21163 *S. dinganii* ET	0.126	—	1	7	8	6	7	9	8	11	13
AK 21223 *S. dinganii* ET	0.126	0.001	—	8	9	7	8	10	7	10	14
SP 5084 *S. dinganii* KE	0.131	0.004	0.004	—	1	1	2	2	1	4	14
SP 5051 *S. dinganii* KE	0.134	0.004	0.005	0.001	—	2	3	3	2	5	15
SP 5077 *S. dinganii* KE	0.129	0.003	0.004	0.001	0.001	—	3	3	2	5	13
TM 37656 *S. dinganii* SA	0.131	0.004	0.004	0.001	0.002	0.002	—	4	3	6	14
SP 10179 *S. dinganii* G	0.127	0.005	0.005	0.001	0.002	0.002	0.002	—	3	5	16
SP 10180 *S. dinganii* G	0.127	0.004	0.004	0.001	0.001	0.001	0.002	0.002	—	3	15
SP 10181 *S. dinganii* G	0.127	0.006	0.005	0.002	0.003	0.003	0.003	0.003	0.002	—	18
MVZ 186412 *S. heathi* VI	0.133	0.007	0.007	0.007	0.008	0.007	0.007	0.008	0.008	0.010	—
MVZ 176513 *S. heathi* CH	0.132	0.006	0.006	0.006	0.007	0.006	0.006	0.007	0.007	0.009	0.001
ROM 110843 *S. kuhlii* VI	0.130	0.018	0.019	0.017	0.018	0.017	0.017	0.018	0.018	0.020	0.019
AK 21490 *S. kuhlii* MA	0.132	0.019	0.020	0.018	0.019	0.018	0.018	0.020	0.019	0.021	0.020
AK 21493 *S. kuhlii* MA	0.131	0.018	0.019	0.017	0.018	0.017	0.017	0.019	0.019	0.020	0.020
TM 37672 *S. viridis* SA	0.132	0.002	0.002	0.003	0.004	0.003	0.003	0.004	0.004	0.005	0.006
SP 10628 *S. nux* C	0.127	0.014	0.014	0.014	0.014	0.012	0.014	0.014	0.014	0.016	0.018
SP 10137 *S. leucogaster* G	0.130	0.010	0.011	0.011	0.012	0.011	0.011	0.012	0.012	0.013	0.013
SP 10136 *S. leucogaster* G	0.130	0.011	0.011	0.012	0.012	0.011	0.012	0.013	0.011	0.013	0.014
SP 5505 *S. nigrita* K	0.124	0.003	0.003	0.004	0.004	0.003	0.004	0.004	0.004	0.006	0.007
SMG 14474 *S. sp.*	0.131	0.006	0.007	0.007	0.007	0.006	0.007	0.008	0.007	0.009	0.009
M. tricolor	0.126	0.202	0.202	0.203	0.203	0.203	0.203	0.203	0.203	0.203	0.206

Table 9. continued

	12	13	14	15	16	17	18	19	20	21	22
E. serotinus	121	118	120	119	121	113	123	123	118	121	115
AK 21163 *S. dinganii* ET	11	33	35	34	3	25	20	21	6	12	173
AK 21223 *S. dinganii* ET	12	34	36	35	4	26	21	20	6	13	173
SP 5084 *S. dinganii* KE	12	32	34	33	6	27	21	22	7	13	174
SP 5051 *S. dinganii* KE	13	33	35	34	7	25	22	23	8	14	174
SP 5077 *S. dinganii* KE	11	31	33	32	5	23	20	21	6	12	174
TM 37656 *S. dinganii* SA	12	32	34	33	6	27	21	22	7	13	174
SP 10179 *S. dinganii* G	14	34	36	35	8	26	23	24	8	15	174
SP 10180 *S. dinganii* G	13	33	35	34	7	25	22	21	7	14	174
SP 10181 *S. dinganii* G	16	36	38	37	10	28	25	24	10	17	174
MVZ 186412 *S. heathi* VI	2	37	39	38	12	34	25	26	12	17	176
MVZ 176513 *S. heathi* CH	—	35	37	36	10	32	23	24	10	15	174
ROM 110843 *S. kuhlii* VI	0.018	—	3	1	31	38	38	39	29	31	177
AK 21490 *S. kuhlii* MA	0.019	0.002	—	4	33	40	40	41	31	33	178
AK 21493 *S. kuhlii* MA	0.019	0.001	0.002	—	32	39	39	40	30	32	178
TM 37672 *S. viridis* SA	0.005	0.016	0.017	0.017	—	27	19	20	5	11	174
SP 10628 *S. nux* C	0.017	0.020	0.021	0.021	0.014	—	31	32	23	24	165
SP 10137 *S. leucogaster* G	0.012	0.021	0.022	0.021	0.010	0.017	—	1	16	12	173
SP 10136 *S. leucogaster* G	0.013	0.021	0.022	0.022	0.011	0.018	0.000	—	16	13	173
SP 5505 *S. nigrita* K	0.006	0.017	0.018	0.017	0.003	0.013	0.009	0.009	—	9	170
SMG 14474 *S. sp.*	0.008	0.016	0.017	0.017	0.006	0.013	0.006	0.007	0.005	—	172
M. tricolor	0.203	0.206	0.207	0.207	0.204	0.196	0.203	0.203	0.198	0.201	—

A maximum parsimony analysis of the 42 haplotypes resulted in 14 equally parsimonious trees at 1305 steps. The tree description for one of the 14 trees is as follows: consistency index = 0.5824, homoplasy index = 0.4176, rescaled consistency index = 0.4989, and retention index = 0.8567 (Fig. 10). The maximum parsimony majority rule bootstrap consensus cladogram identified 14 clades (Fig. 11). Bootstrap support values over 50% are labeled on the appropriate nodes while nodes with less than 50% bootstrap support are collapsed. The topology shows *S. kuhlii* as the most basal *Scotophilus*. The African *Scotophilus* comprise a monophyletic clade. One Asian species, *S. heathi*, is within the African clade and sister to an undescribed *Scotophilus*

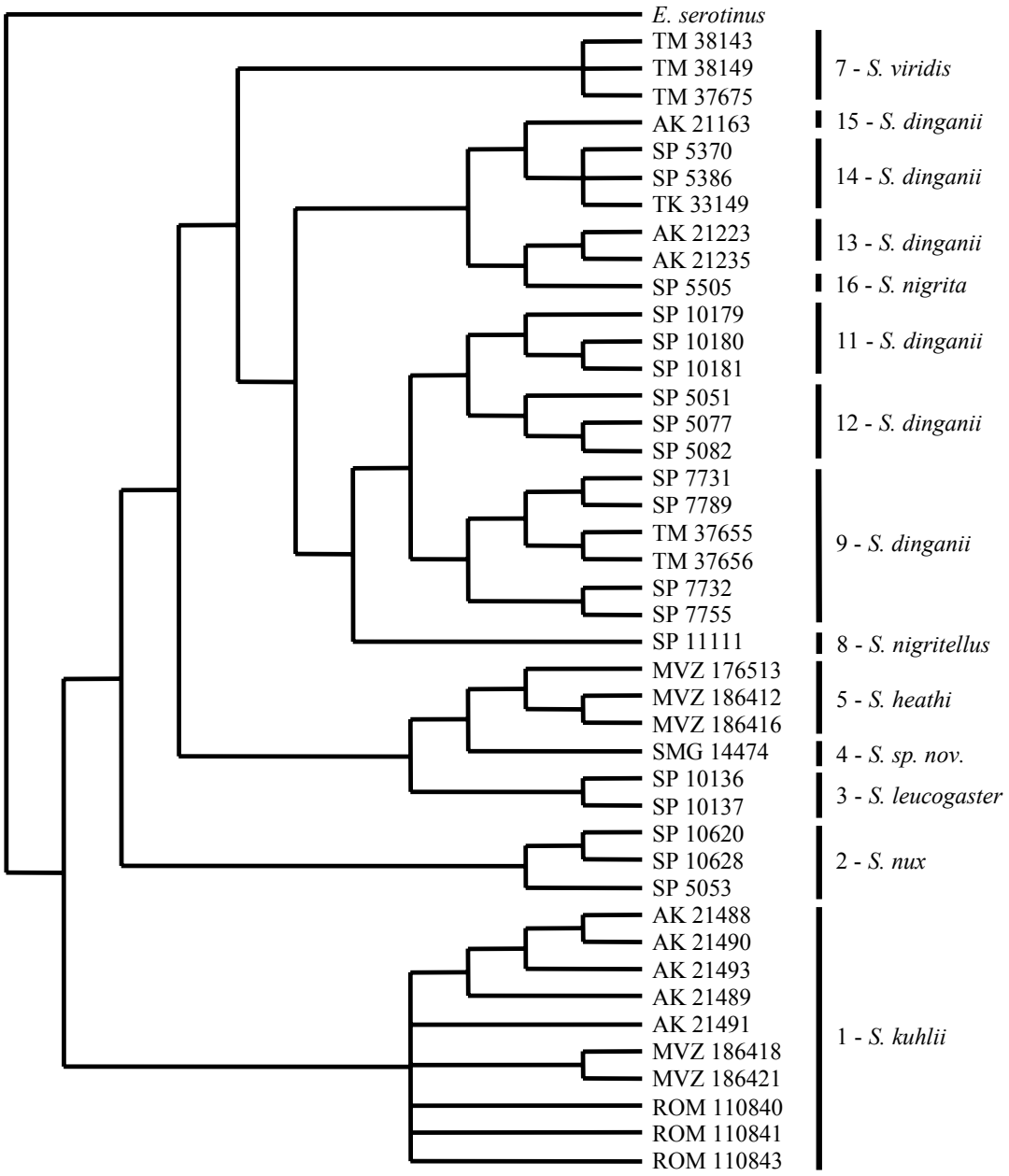


Figure 10. The strict consensus tree of 22 equally parsimonious cladograms of 42 combined dataset haplotypes. The tree was inferred using Maximum Parsimony optimaltiy criteria in PAUP. Geographic localities of the 14 major tip clades are as follows: 1 = Vietnam, Malaysia; 2 = Kenya, Cameroon; 3 = Ghana; 4 = Madagascar; 5 = Vietnam, China; 8 =Ghana; 7 = South Africa; 9 = South Africa; 11 = Ghana; 12 = Kenya; 16 = Kenya; 13 = Ethiopia; 14 = Kenya; 15 = Ethiopia;. The outgroup taxon is *Eptesicus serotinus*.

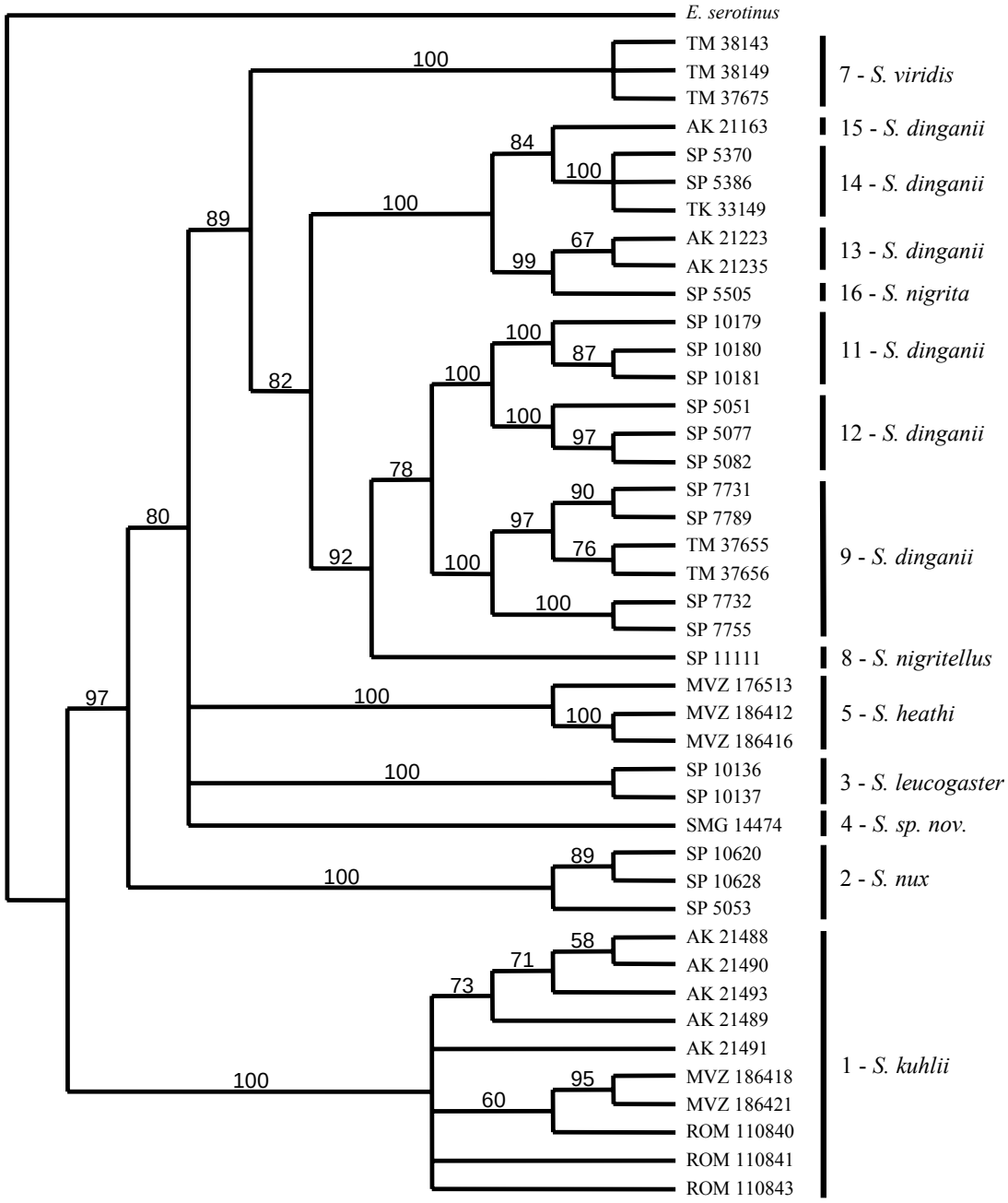


Figure 11. Maximum parsimony bootstrap consensus cladogram of 42 haplotypes representing nine species of *Scotophilus* constructed in PAUP. Geographic localities of the 14 major clades are as in figure 10. The outgroup taxon is *Eptesicus serotinus*.

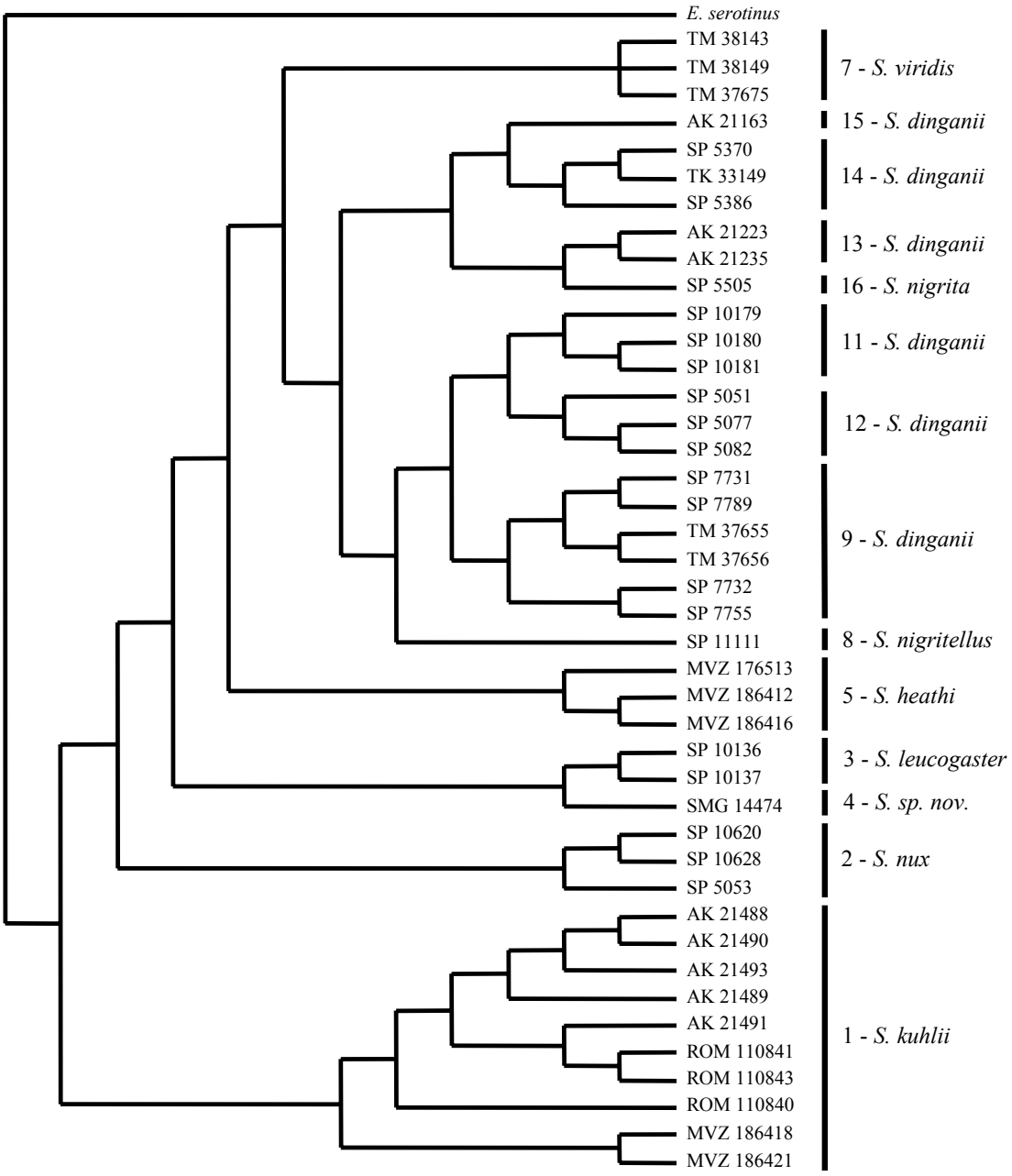


Figure 12. Maximum likelihood (-Ln = 11030.59) cladogram of 42 combined data set haplotypes. The cladogram was constructed in PAUP using the GTR + Γ + I model of nucleotide substitution. Geographic localities of the 14 major clades are as in figure 10. The outgroup taxon is *Eptesicus serotinus*.

from Madagascar in figure 10. Of the African taxa, *S. nux* (clade 2) occupies the most basal position on the cladogram. The large African taxon, *S. nigrita* (clade 10), is grouped with a clade containing Ethiopian *S. dinganii* (clade 11). Within the monophyletic clade containing *S. dinganii*, several lineages of *S. dinganii* are present. The *S. dinganii* clade is composed of two major clades, one containing *S. dinganii* from Ethiopian and Kenyan localities and the other clade containing *S. dinganii* from Ghana, a single locality in Kenya (Kakamega forest), and South Africa. Within these two major clades, extensive geographic subdivision is present based on locality.

A maximum likelihood (ML) analysis of the combined data set was accomplished in PAUP. The program MODELTEST selected the GTR + Γ + I model as the best fit model of nucleotide substitution. Nucleotide frequencies were set to A = 0.29740, C = 0.21630, G = 0.17080, and T = 0.31550. The gamma shape parameter was set to $\alpha = 0.5120$ and the assumed proportion of invariable sites was set to 0.6417. The ML analysis resulted in a maximum likelihood tree with a score of –Ln likelihood = 11030.59 (Fig. 12). The ML bootstrap consensus tree is displayed in figure 13. The topology shows the same 14 clades being recovered as in the MP tree (Fig. 11) but several of the internal nodes are collapsed with no resolution present for the placement of several groups. A clade containing all African taxa except for *S. nux* is weakly supported with a bootstrap support value of 59%.

The Bayesian analysis of the combined data matrix under the GTR + Γ + I model of nucleotide substitution produced a phylogeny (Fig. 14) identical to the MP phylogeny (Fig. 10) with respect to the arrangement of the African clade. The three independent

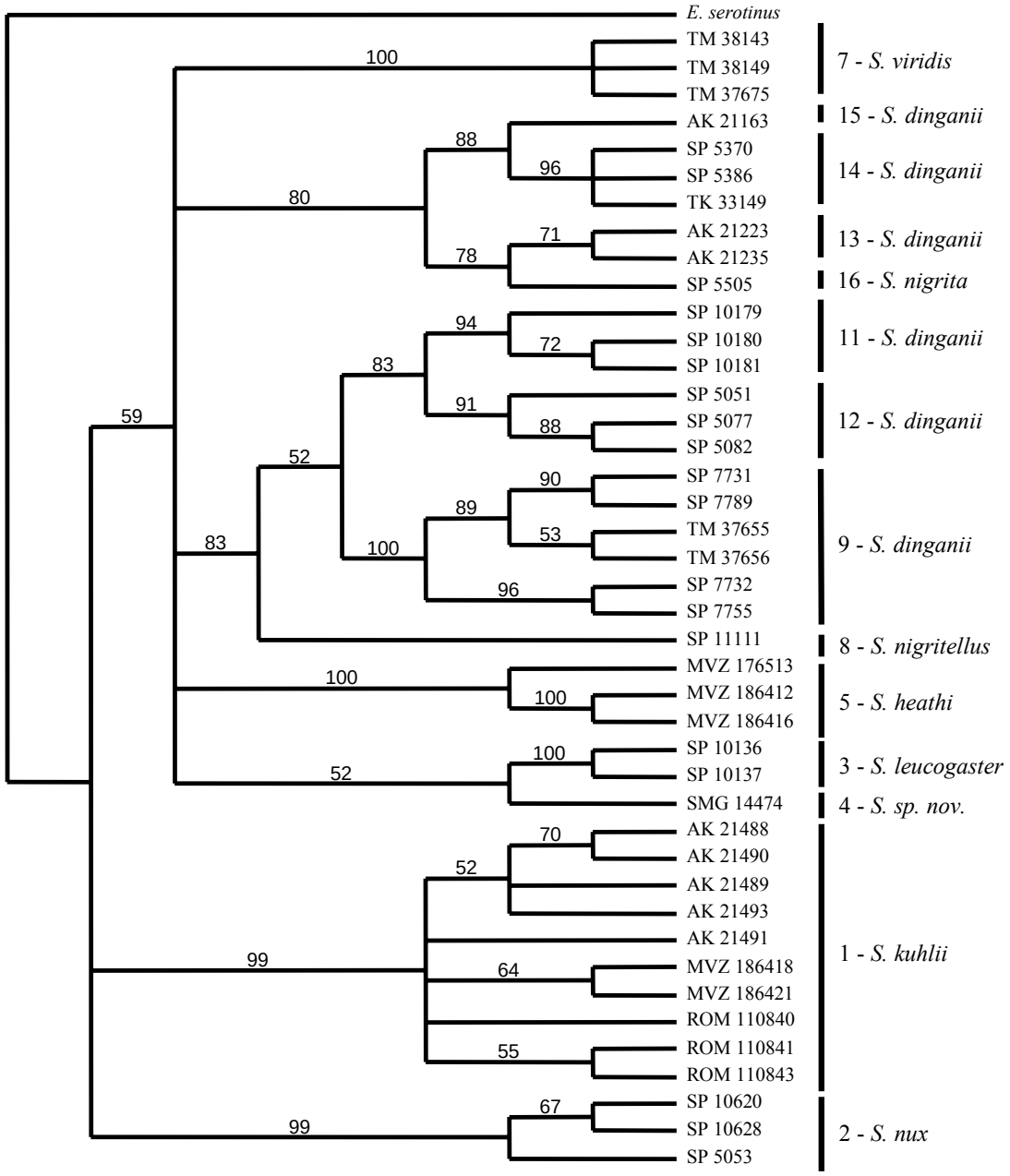


Figure 13. Maximum likelihood bootstrap consensus cladogram of 42 combined dataset haplotypes. The cladogram was constructed in PAUP using the GTR + I + Γ model of nucleotide substitution. The numbers above the nodes are bootstrap support values. Geographic localities of the 14 major tip clades are as in figure 10. The outgroup taxon is *Eptesicus serotinus*.

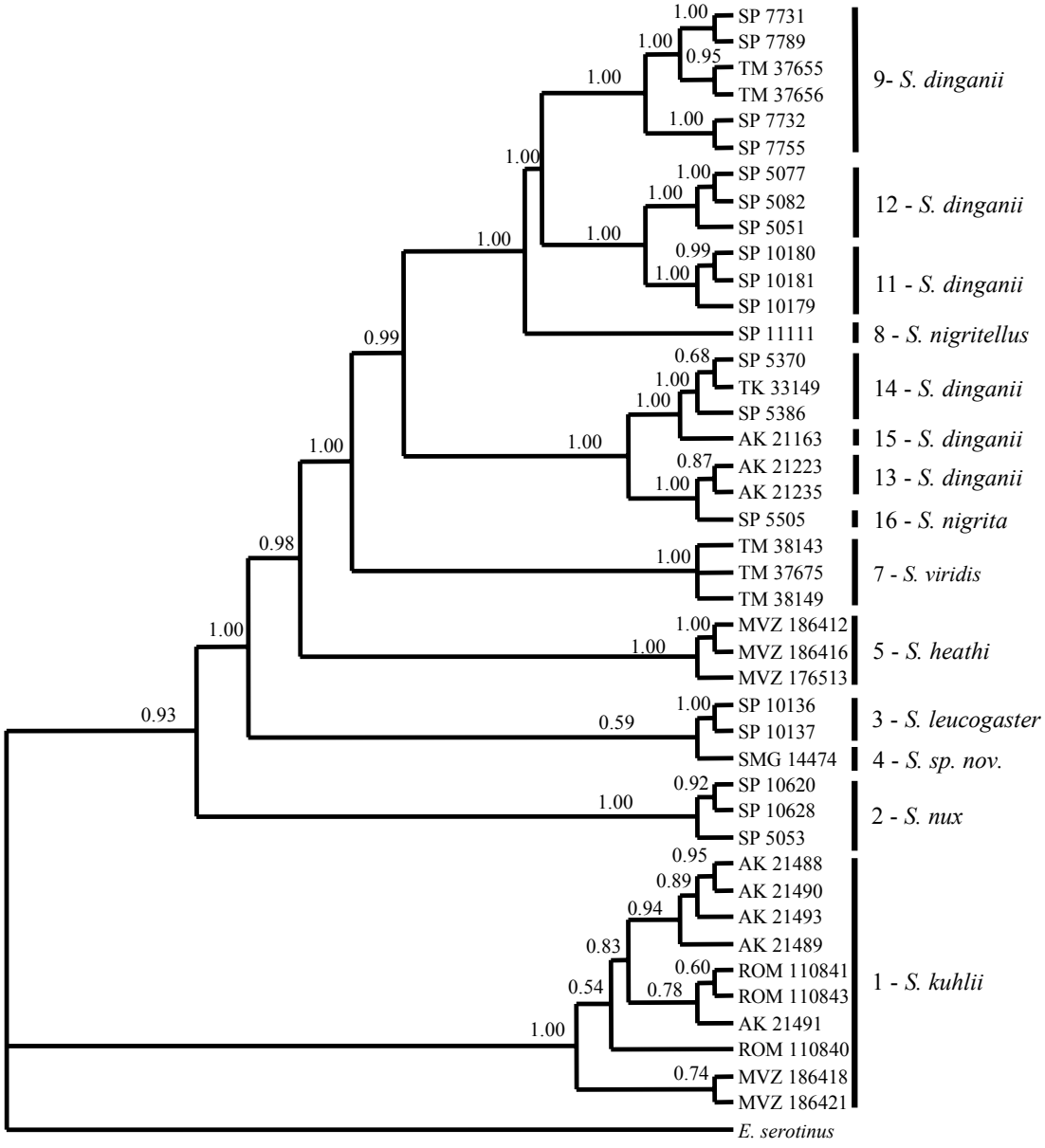


Figure 14. The 50% majority rule consensus tree from a Bayesian analysis of 42 combined dataset haplotypes. The tree was computed in Mr. Bayes using the GTR + I + Γ model of nucleotide substitution. Posterior probability support values are listed above the nodes. Geographic localities of the 14 major clades are as in figure 10. The outgroup taxon is *Eptesicus serotinus*.

analyses converged on stable posterior probability values after a burn in time of 30,000 generations. The nucleotide substitution model parameter estimates (GTR + Γ + I) are given in table 10. The phylogeny inferred has a log likelihood score of –Ln = 11065.86 and shows high posterior probability support for the same 14 tip clades as recovered in the MP and ML analysis, and unlike the ML tree, no internal nodes are collapsed for lack of support.

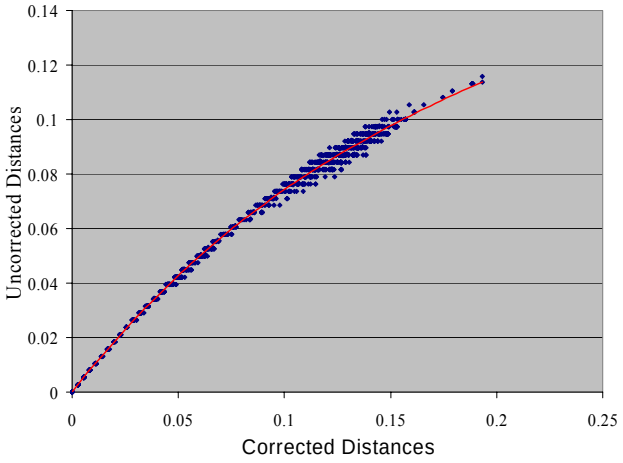
Table 10. Nucleotide substitution parameter estimates under the GTR + Γ + I model from a Bayesian analysis of the combined data sets.

	Mean	Variance	95%CI		Median
TL	1.081589	0.006534	0.933000	1.241000	1.081000
r_{GT}	1.000000	0.000000	1.000000	1.000000	1.000000
r_{CT}	20.665293	23.160209	13.602631	32.364057	19.901253
r_{CG}	0.538126	0.077086	0.151522	1.178711	0.488024
r_{AT}	0.758148	0.055137	0.412239	1.343131	0.722479
r_{AG}	10.723398	6.333424	7.060134	17.353959	10.300934
r_{AC}	1.740172	0.218183	1.044524	2.871380	1.670631
π_A	0.299680	0.000054	0.285422	0.314223	0.299558
π_C	0.224626	0.000044	0.211956	0.237492	0.224639
π_G	0.165401	0.000036	0.153415	0.176995	0.165305
π_T	0.310294	0.000054	0.296494	0.324894	0.310229
α	0.404099	0.009038	0.261149	0.630740	0.390365
Pinvar.	0.512187	0.002838	0.400993	0.609542	0.514956

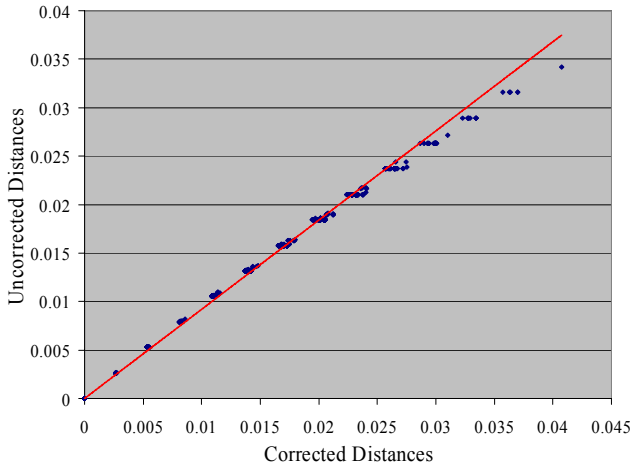
Saturation Plots

Plots of uncorrected versus corrected genetic distances were constructed to explore the level of saturation present in the cytochrome *b* dataset. The plot of the individual cytochrome *b* codon positions are shown in figure 15. A saturation plot of the entire cytochrome *b* gene along with a plot showing the relative contribution of each

A.



B.



C.

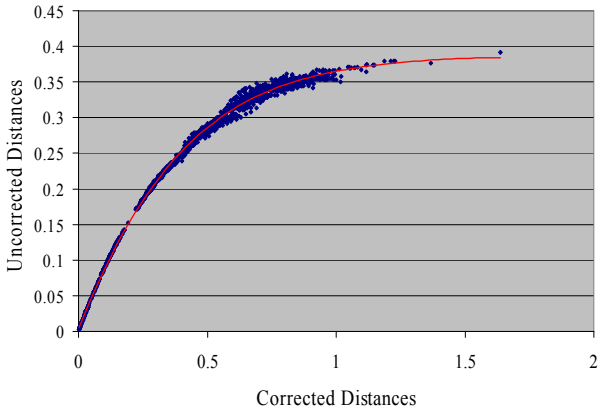


Figure 15. Saturation plots for the three cytochrome *b* codon positions. A. = position 1 plot, B. = position 2 plot, and C. = position 3 plot

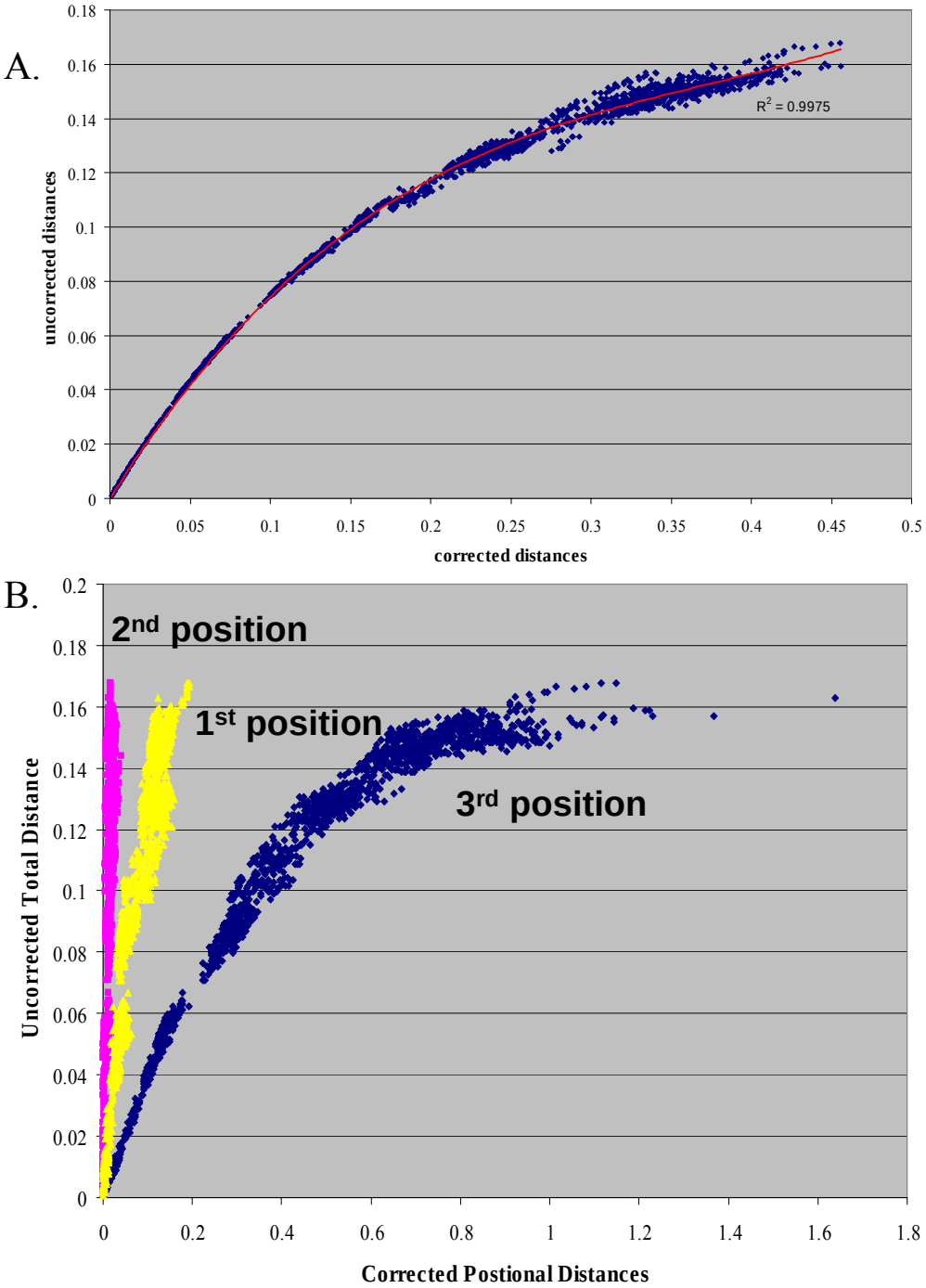


Figure 16. Saturation plots of the complete cytochrome *b* gene. Figure 16 A is a plot of the complete gene and fgure 16 B is a plot showing the positional contributions to the plot of the entire gene.

codon position to the plot of the entire cytochrome *b* gene is presented in figure 16.

A plot of pairwise comparisons of genetic distance based on the cytochrome *b* data and *zfy* data is presented in figure 17. The scales present on the x and y axisis account for an approximately ten fold difference in mutation rates between the cytochrome *b* gene and the *zfy* gene.

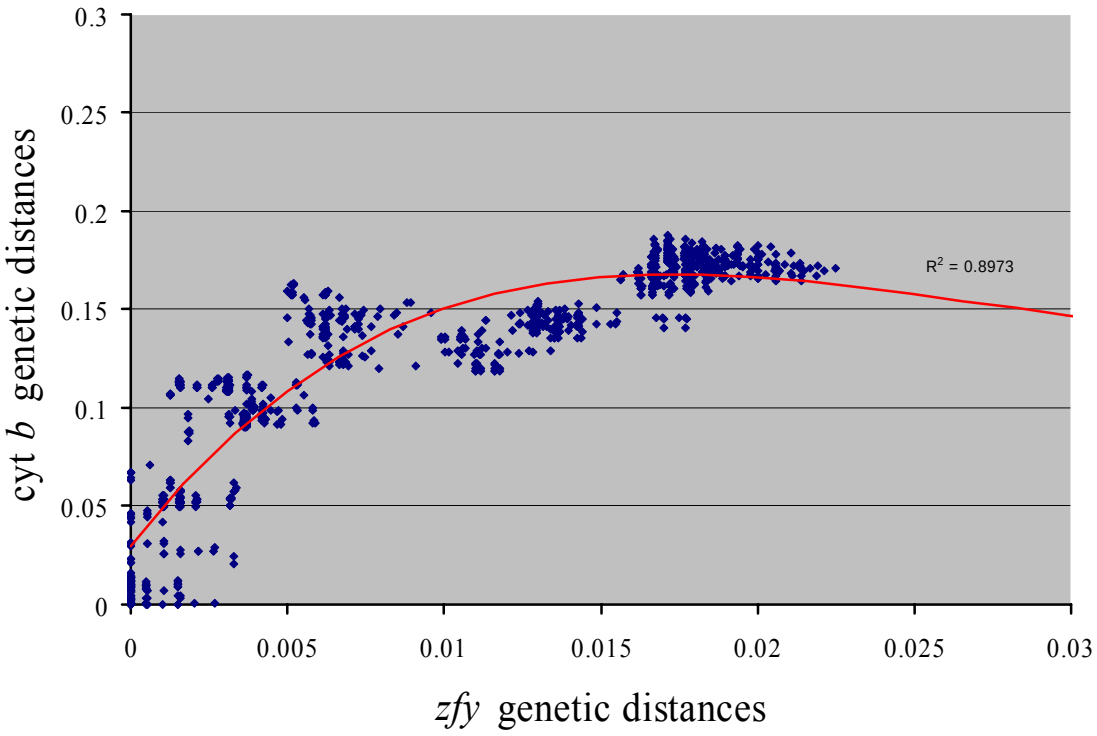


Figure 17. Plot of pairwise genetic distance for the cytochrome *b* gene and the region of the *zfy* gene sequenced in this study.

DISCUSSION

How Many *Scotophilus* Species Exist?

The number of *Scotophilus* species in existence has been a contentious and confusing affair as realized through a review of the systematic and taxonomic literature related to this genus. Robbins et al. (1985) recognized six species of *Scotophilus* occurring in sub-Saharan Africa: *S. dinganii*, *S. leucogaster*, *S. nigrita*, *S. nucella*, *S. nux*, and *S. viridis*. This conclusion has been followed in the most recent account of the species that is presented by Simmons (in press). Fourteen recognized species of African and Asian *Scotophilus* are listed as follows: *S. borbonicus* (E. Geoffroy, 1803); *S. celebensis* Sody, 1928; *S. collinus* Sody 1936; *S. dinganii* (A. Smith, 1833); *S. heathi* (Horsfield, 1831); *S. kuhlii* Leach, 1821; *S. leucogaster* (Cretzschmar, 1830); *S. nigrita* (Schreber, 1774); *S. nigritellus* de Winton, 1899; *S. nucella* Robbins, 1983 ; *S. nux* Thomas, 1904; *S. robustus* Milne-Edwards, 1881; *S. sp. nov.* Goodman et al, in press; and *S. viridis* (Peters, 1852). Recent additions to the list of species are *S. collinus* (Kitchener et al., 1997), which was removed from the synonomy of *S. kuhlii* and raised to full species based on morphological differences, and *S. sp. nov.* (Goodman et al., in press), which is currently being described from Madagascar. This study is the first to address the taxonomy of *Scotophilus* with use of molecular data.

Phylogenies produced by the cytochrome *b* data set resulted in 15 clades identified based on field/voucher identifications and localities. These 15 clades are as follows: 1 = *S. kuhlii* from Vietnam, Malaysia, Philippines; 2 = *S. nux* from Kenya and Cameroon; 3 = *S. leucogaster* from Ghana; 4 = *S. sp.* is a new species from Madagascar

currently being described based on morphology (Goodman et al., in press); 5 = *S. heathi* from Vietnam, China; 6 = *S. robustus* from Madagascar; 7 = *S. viridis* from South Africa; 8 = *S. nigritellus* from Ghana; 9 = *S. dinganii* from South Africa; 10 = *S. viridis* from Kenya; 11 = *S. dinganii* from Ghana; 12 = *S. dinganii* from Kenya; 13 = *S. dinganii* from Ethiopia and 1 *S. nigrita* from Kenya; 14 = *S. dinganii* from Kenya; and 15 = *S. dinganii* from Ethiopia and Kenya. These 15 clades show pairwise estimates of sequence divergence ranging from 3% to >16% (table 5). Based on the cytochrome *b* phylogenies (Figs. 4, 6, and 7) and the sequence divergence values in table 5, there is support for 13 species of *Scotophilus*. Clades 1, 2, 3, 4, 5, and 6 each represent distinct species with all clades forming their own unique highly supported monophyletic groups.

Individuals that were identified as *S. viridis* occur in two very divergent clades, suggesting the occurrence of two species groups among *S. viridis sensu stricto*. *S. viridis* was described originally based on material collected in coastal Mozambique, and clade 7 is composed of South African individuals identified as *S. viridis*. Clade 10 is composed of six individuals identified as *S. viridis* from Kenya and is sister to clade 9, which represents *S. dinganii* from South Africa. These sister clades fall within a larger group of *S. dinganii* individuals from Ghana and the forests of western Kenya. Therefore, either this sample of *S. viridis* from Kenya is *S. dinganii* or it is a new species of *Scotophilus*. Prelimenary examination of voucher material suggests that this group is not *S. dinganii* but rather has morphological similarities to *S. viridis*.

Clade 8 includes eight individuals of *S. nigritellus* from Ghana. This clade and its position on the phylogeny provides support for the recognition of *S. nigritellus* as a

distinct species of *Scotophilus* and is divergent enough from individuals identified as *S. viridis* from South Africa or Kenya to merit species recognition. This result supports the taxanomic conclusion by Grub et al. (1998).

Clade 13 includes an individual identified as *S. nigrita* (SP 5505) from Kenya which clusters with *S. dinganii* from Ethiopia. The possibility that this individual was not properly identified is remote as the size difference between *S. nigrita* and *S. dinganii* make the two species easily identifiable. This association shows that *S. nigrita* shares a cytochrome *b* haplotype that is very similar to those found in Ethiopian *S. dinganii*. This could suggest a mitochondrial capture event in the evolutionary history of this individual potentially due to a hybridization event. This will be discussed further in detail after discussion of all the data.

Clades 13, 14, and 15 represent 27 individuals identified as *S. dinganii* that share closely related cytochrome *b* haplotypes. The amount of sequence divergence between these clades is from 3-5%. This range has been suggested as a divergence range seen between closely related species (Bradley and Baker, 2001). The individuals in these clades are from Ethiopia and northern and eastern savannah localities in Kenya. Together they share a single common ancestor and as such represent one species of *S. dinganii* from Ethiopia and Kenya.

Individuals identified as *S. dinganii* also occur in clades 9, 11, and 12. Clade 9 includes *S. dinganii* from northeastern South Africa, clade 11 contains *S. dinganii* from coastal Ghana, and clade 12 contains *S. dinganii* from the forests of western Kenya. These clades differ from each other by 3-5% sequence divergence and differ from clades

13, 14, and 15 by 9 – 10% sequence divergence. Clearly these clades represent another distinct species of *Scotophilus* sufficiently divergent from the Ethiopian and Kenyan *S. dinganii* to warrant such recognition and may well represent three cryptic species of *S. dinganii* as a clade of *S. viridis* from Kenya falls within this clade.

The phylogenies based on the *zfy* dataset resulted in 9 major clades (Figs. 8 and 9), again identified based on field/voucher identifications and localities. These 9 major clades are numbered as in the cytochrome *b* clades and are as follows: 1= *S. kuhlii* from Vietnam and Malaysia; 2 = *S. nux* from Cameroon and Kenya; 3 = *S. leucogaster* from Ghana; 4 = a new species from Madagascar; 5 = *S. heathi* from Vietnam and China; 7 = *S. viridis* from South Africa; a clade composed of *S. dinganii* from Ghana, South Africa, and Kenya that represents cytochrome *b* clades 9, 11, and 12; a clade composed of *S. dinganii* from Ethiopia and Kenya that represents cytochrome *b* clades 13 and 15; and a clade composed of *S. nigrita* from Kenya labeled as clade 16 (this specimen was within clade 13 in the cytochrome *b* phylogenies). This latter observation suggests incongruence between the cytochrome *b* and *zfy* phylogenies (Figs. 4 and 8). The 9 clades show pairwise sequence divergence values that range from 0.2% to 2% between the tip clades (table 9) for the *zfy* data. These values of *zfy* divergence are similar to those reported by Wallner et al. (2003) in their paper on *Equus* species and by Lawson and Hewitt (2001) for several sheep and goat species.

The *zfy* data provide support for species designations based on the cytochrome *b* dataset. Clades 1, 2, 3, 4, 5, and 7 each represent *Scotophilus* species as identified with the cytochrome *b* data. A clade composed of *S. dinganii* from Kenya, South Africa, and

Ghana represents the *S. dinganii* found in clades 9, 11, and 12 of the cytochrome *b* phylogenies and provides further evidence for the recognition of these individuals as conspecific. Individuals in these clades share closely related mitochondrial haplotypes as well as closely related *zfy* haplotypes. A *zfy* clade representing *S. viridis* individuals from South Africa corresponds to clade 7 seen in the cytochrome *b* dataset. A clade representing individuals from Ethiopia and Kenya corresponds to clades 13, 14, and 15 in the cytochrome *b* data, again providing support for species recognition of these individuals as distinct from the other group of *S. dinganii* from Kenya, Ghana, and South Africa. A new clade, clade 16 in the *zfy* phylogenies, represents *S. nigrita*, which has a very distinct *zfy* haplotype compared to Ethiopian *S. dinganii* with which it clusters in the mtDNA trees. This again provides some evidence for a mitochondrial capture event in the history of this species possibly resulting from interspecific hybridization with a *S. dinganii*-like ancestor.

The combined phylogenies resulted in 14 clades (Figs. 10, 11, 12, 13 and 14). These 14 clades are numbered as in the cytochrome *b* and *zfy* clades and are as follows: 1 = *S. kuhlii* from Vietnam and Malaysia; 2 = *S. nux* from Kenya and Cameroon; 3 = *S. leucogaster* from Ghana, 4 = a new species from Madagascar, 7 = *S. viridis* from South Africa; 8 = *S. nigritellus* from Ghana; 9 = *S. dinganii* from South Africa; 11 = *S. dinganii* from Ghana; 12 = *S. dinganii* from Kenya; 13 = *S. dinganii* from Ethiopia; 14 = *S. dinganii* from Kenya; 15 = *S. dinganii* from Ethiopia; and 16 = *S. nigrita* from Kenya.

Based on the phylogenies inferred from mtDNA, *zfy*, and the combined data set 16 species of *Scotophilus* are identified (Table 11). The species identified in this study

are as follows: clade 1 = *S. kuhlii*; clade 2 = *S. nux*; clade 3 = *S. leucogaster*; clade4 = *S. sp. nov.*, a new species from Madagascar (Goodman et al., in press); clade 5 = *S. heathi*; clade 6 = *S. robustus*; clade 7 = *S. viridis*; 8 = *S. nigritellus*; clades 9, 11, and 12 = *S. dinganii*; clade 10 = *S. viridis* (Kenya); clades 13, 14, and 15 = *S. dinganii* (eastern Africa); and clade 16 = *S. nigrita*. I agree with Simmons (in press) and Robbins et al. (1985) in restricting *S. borbonicus* to Reunion Island and Madagascar. The species

Table 11. The sixteen species of *Scotophilus* recognized in this study. A + indicates support and a - indicates a lack of support based on either morphology, mtDNA or Y DNA.

Species	Morphology	mtDNA	Y DNA
S. borbonicus (E. Geoffroy, 1803)	+	no data	no data
S. celebensis Sody, 1928	+	no data	no data
S. collinus Sody, 1936	+	no data	no data
S. dinganii (A. Smith, 1833)	+	+	+
S. dinganii (eastern Africa)	no data	+	+
S. heathi (Horsefield, 1831)	+	+	+
S. kuhlii Leach, 1821	+	+	+
S. leucogaster (Cretzschmar, 1830)	+	+	+
S. nigrita (Schreber, 1774)	+	-	+
S. nucella Robbins, 1973	+	no data	no data
S. nux Thomas, 1904	+	+	+
S. robustus Milne-Edwards, 1881	+	+	no data
S. sp. nov. Goodman et al., in press	+	+	+
S. viridis (Peters, 1852)	+	+	+
S. viridis (eastern Africa)	no data	+	no data
S. nigritellus	no data	+	no data

identified in this study along with the *Scotophilus* species not present in this study (*S. celebensis*, *S. collinus*, *S. borbonicus*, and *S. nucella*) result in a total of 16 species of *Scotophilus*.

The two new species proposed by this study along with the confirmation of the specific status of *S. nigritellus* and confirmation of a newly described Malagasy species are defined based on cytochrome *b* data, *zfy* data, and/or a combination of the two datasets. There is a growing literature on cryptic species and their definition based on genetic data. Such examples exist for the Onychophora (Trewick, 1998), birds (Baker et al., 1995) and mammals (Kingston et al., 2001; Olson et al., 2004). The use of mtDNA to define species was investigated by Bradley and Baker (2001) in relation to mammals and used as a test of the genetic species concept. The use of mtDNA in conjunction with nuclear DNA markers allows for a more robust definition of species based on nucleotide sequences. The results of Bradley and Baker (2001) indicated that cytochrome *b* genetic distance values between 2% - 11% were indicative of probable species and that distances above 11% were indicative of species recognition. In the genus *Scotophilus*, known morphological species differ from other known morphological species by as little as 4% sequence divergence up to over 16% sequence divergence. The two new species identified differ by 5% or more sequence divergence from recognized species groups. The two cryptic *S. dinganii* species, *S. dinganii* and *S. dinganii* (eastern Africa) differ by 5% sequence divergence for cytochrome *b* (Table 5) and also by 0.4% sequence divergence for *zfy* (Table 9). The *zfy* divergence value is one that has been reported as a range between *Equus* species (Wallner et al., 2003). The *S. viridis* species identified, *S.*

viridis eastern Africa, also differs by more than 5% sequence divergence from recognized *S. viridis*-like species groups. *S. viridis* (eastern Africa) and *S. nigritellus* differ by 6% while both of these differ by more than 10% from *S. viridis* (Table 5). Robbins et al. (1985) noted that southern African *S. viridis* were slightly larger and exhibited greater size variation than other *S. viridis* in their study. The new Madagascan species, *S. sp. nov.*, has been described morphologically by Goodman et al. (in press) and is supported by the molecular data in this study. This Madagascan endemic differs from other *Scotophilus* species by 13% or greater sequence divergence.

The two new species identified in this study should next be validated with morphology and/or other ecological data. A recent abstract in *African Bat Conservation News* by Jacobs et al. (2004) reports sympatric species of *S. dinganii*-like bats in southern Africa based on cytochrome *b* sequence data (3.4% sequence divergence) and echolocation frequency data (peak echolocation frequencies of 44 kHz for one and 33 kHz for the other). These data together with this study provide evidence that *S. dinganii* could possibly be a species complex composed of species that are morphologically indistinguishable but clearly distinct genetically. Ironically, *S. dinganii* is abundant and relatively easy to catch. Both of these qualities should make answering this question relatively simple. This result has several implications for *Scotophilus* taxonomy and systematics. First, it signals that call frequency data may be a useful phylogenetic marker for separating species. Second, it opens the possibility for the widespread *S. dinganii* to be a complex of several closely related species that deserves further inquiry.

In any case, the common yellow house bats are proving to be a very interesting group of species that hold the potential to increase our knowledge of cryptic species.

Scotophilus Phylogeny

An understanding of the phylogenetic relationships among species of the genus *Scotophilus* is the result of the phylogenies produced under ML, MP and Bayesian methods based on both cytochrome *b* and Y chromosome datasets. In all analyses and with both datasets, the following phylogenetic observations are supported: *S. kuhlii* is found to be the most basal taxon; African members of *Scotophilus* form a monophyletic group; the two Asian taxa analyzed in this study share a distant relationship and do not share a nearest common ancestor; the two Madagascan species in this study do not form a sister group relationship suggesting multiple origins of Malagasy *Scotophilus*; *S. nux* is the most basal taxon in the African clade (although this is not supported in the ML phylogeny inferred from the combined dataset); *S. nigritellus* is distinct from *S. viridis* (eastern Africa) and *S. viridis* and shares a sister group relationship with a *S. dinganii* clade from Kenya, Ghana, and South Africa; at least two *S. viridis*-like taxa are present on mainland Africa; northern and eastern Kenyan *S. dinganii* share a close relationship with Ethiopian *S. dinganii*; and *S. dinganii* from western Kenya, coastal Ghana, and northeastern South Africa share a close relationship.

Support for other phylogenetic relationships is observed in the MP, ML, Bayesian, or a combination of the three methods of data analysis. There is weak support for a sister group relationship between *S. robostus* and *S. viridis* based on MP and ML cytochrome *b* data (Figs. 4 and 6) and strong support for this same relationship based on

a Bayesian analysis of cytochrome *b* data (Fig. 7). The *zfy* data set shows support for a sister-group relationship between individuals identified as *S. viridis* and *S. dinganii* from Ethiopia, again with weak support for both the MP and ML (Fig. 8) analysis and strong support from the Bayesian analysis (Fig. 9).

The overall view of *Scotophilus* systematic relationships is that *S. kuhlii* is the basal taxon. African *Scotophilus* form a monophyletic group with *S. nux* as the most basal African taxon. The two Indomalayan species share a distant relationship with a potential African origin of *S. heathi* suggesting multiple origins of the Asian *Scotophilus*. The Malagasy *Scotophilus* appear to have affinities with the African species and likely multiple origins from Africa as *S. robustus* and *S. sp. nov.* do not share a sister group relationship.

The large bodied African species, *S. nigrita*, is closely related to *S. dinganii* and in fact forms a sister group relationship with an Ethiopian *S. dinganii* based on mtDNA and a more distant relationship to all *S. dinganii* based on *zfy*. This provides some evidence for a mitochondrial capture event in the history of this species possibly due to a hybridization event with a *S. dinganii*-like ancestor. The occurrence of a similar introgressive hybridization has been reported in North American deer (Cathey et al., 1998). Unfortunately, the sample size of *S. nigrita* available for this study is the single individual sequenced. Additional specimens of *S. nigrita* are needed to be sequenced to further investigate this evolutionary scenario.

The systematic relationships between other members of the genus remain unclear. There is no statistical support for nodes joining several species. The branch

lengths joining the supported nodes are small, suggesting a rapid radiation of *Scotophilus* taxa and subsequent difficulty in obtaining statistical support for several systematic relationships. My results corroborate those reported by Hoofer and Van Den Bussche (2003) based on 12S rRNA, 16S rRNA, and $tRNA^{Val}$ data. Their data indicated a close relationship between Ethiopian species and a distant relationship between the two Indomalayan species (*S. heathi* and *S. kuhlii*).

This study has resulted in several more questions regarding the systematics of the genus *Scotophilus*. Is *S. nigrita* a species that has hybridized with an *S. dinganii*-like ancestor? Are there more than two cryptic species of *S. dinganii*-like bats in Sub-Saharan Africa? The origin of Malagasy *Scotophilus* also needs to be further examined with an increased representation of Malagasy and Asian taxa. Also, a thorough survey of the Asian species (*S. kuhlii* and *S. heathi*) is needed to further characterize these taxa. This study puts forth several testable hypotheses of *Scotophilus* systematics and taxonomy.

Biogeographical Implications

The results of this study lead to some interesting biogeographic observations. The two species from Madagascar do not share a sister-group relationship. This suggests the possibility of multiple origins of the Malagasy *Scotophilus* and since both are within the African clade it would suggest an African origin for Malagasy *Scotophilus*.

In Africa there are 9 species of *Scotophilus* including: *S. dinganii* (eastern Africa), *S. dinganii*, *S. viridis*, *S. viridis* (eastern Africa), *S. nigritellus*, *S. nux*, *S.*

leucogaster, *S. nucella*, and *S. nigrita*. Although no supported conclusion can be made as to the origin of *Scotophilus*, the base of the tree resides in Asia, while the majority of the radiation occurs in Africa. More work is needed to fully understand the origins of the genus and to elucidate the phylogenetic relationships present. From the data, it is clear that *S. heathi* represents an invasion of Asia from Africa as it is well embedded in the African clade and this interpretation is the most parsimonious explanation. One member of the African *Scotophilus*, *S. leucogaster*, has been collected in Yemen and Saudi Arabia. These records could possibly indicate a route between Africa and Asia used by a *Scotophilus*-like ancestor. More data are needed to support or refute this hypothesis.

Of the African species, the two *S. dinganii* species show substantial population subdivision. In *S. dinganii* (eastern Africa), there are three major clades, one clade includes an Ethiopian *S. dinganii* and a Kenyan *S. dinganii* (Clade 15). Another clade is composed entirely of individuals collected from scattered Kenyan localities except for the Kakamega forest specimens (Clade 14). These two clades share a sister-group relationship. The sister-group to these two clades is a group of Ethiopian *S. dinganii* (eastern Africa) and includes *S. nigrita* (Clade 13). These three clades merit additional study as they are all related yet differ from each other by 3% -5% sequence divergence. This is a value that has been suggested to indicate closely related species in mammals (Bradley and Baker, 2001).

In Africa, there are at least two *S. viridis*-like species. These include *S. viridis* from South Africa which is close to the type locality of *S. viridis* and probably represents

true *S. viridis* and the species found in east Africa is *S. viridis* (eastern Africa). The species found in West Africa is *S. nigritellus* which has been listed as a synonym of *S. viridis* by some authors (Robbins et al., 1985; Simmons, in press) but treated as a distinct species by Grub et al. (1998). All three taxa are sufficiently distinct from one another to merit species recognition based on cytochrome *b* sequence data. Further inquiry into *S. viridis* and *S. nigritellus* is needed to establish these species with nuclear DNA data, call frequency data, or morphological data. Also, given the three unique clades, more individuals from throughout the range of these three probable species are needed to define their respective species boundaries.

Available Names for the New Species of African *Scotophilus*

S. dinganii (eastern Africa) includes samples from Ethiopia and northern and eastern Kenya. Koopman (1975) lists a number of available names in his synonomy for *S. nigrita* [= *S. dinganii*] as junior synonyms but recognizable supspecies. At the same time, Koopman (1975) considers *S. leucogaster* as a savannah subspecies of *S. dinganii* and lists the following as synonyms of *S. nigrita* [=*S. dinganii*] *leucogaster*: *S. murino-flavus* (Heuglin, 1861) described originally from Massaua, Eritrea; *S. flavigaster* (Heuglin, 1861) described originally from Keren, Eritrea; and *S. serratus* (Heuglin, 1861) originally described from Arashkol, Sudan. All of these were listed as synonyms of *S. leucogaster*.

The next available name is one proposed by Thomas (1904) and listed as *S. nigrita* [=*S. dinganii*] *colias* by Koopman (1975). This was a valid subspecies of *S. dinganii* from the savannahs of central Kenya, originally described from Fort Hall,

Kenya. This is within the physiographic region of Kenya from which my samples were obtained and so this name would be available for the taxon referred to as *S. dinganii* eastern Africa.

The clade representing the *S. viridis* eastern Africa samples are distinct from the South African clade representing true *S. viridis*. Although *S. viridis* was originally described from Mozambique Island, the northeast South African samples are from physiographically uniform habitat and are considered at this time to represent *S. viridis*. For the eastern African samples of *S. viridis*-like bats, three nomenclatural options exist. First, I have followed Robbins et al. (1985) and Simmons (in press) in regarding the species *S. borbonicus* to be confined to Reunion Island and not occurring on mainland Africa. If however, this species is found to occur on mainland Africa, it might be the available name for samples from eastern Africa.

A second option is the availability of a previously proposed name already in synonomy. A review of the literature with synonyms listed for all species of *Scotophilus* from eastern and northeastern Africa did not reveal any available names that were readily recognizable as available for eastern African *S. viridis*-like bats. As indicated above, available names are listed as synonyms of *S. leucogaster* or *S. dinganii*. Only *S. damarensis* Thomas, 1906, (originally described from Namibia) is listed as a synonym of *S. viridis* and so might be an available name for the eastern African samples of *S. viridis*-like bats.

The third option for assigning a species name on the eastern African *S. viridis*-like samples is to propose a new name for the bats in these clades. Before doing so, a

careful review of the literature and morphometric comparisons of the vouchers for the tissues must be done with properly identified comparison material so as to be certain an unnecessary synonym is not proposed as a new name.

Heterogenous Rates of Nucleotide Substitution

The plot pairwise comparisons of genetic distance based on the cytochrome *b* data and *zfy* data presented in figure 17 shows the differential rates of nucleotide substitution present in these two genes. A one to one relationship would be expected with most data points plotting along a 45º line if the cytochrome *b* gene and the *zfy* gene were evolving at a similar rate. This is not the case, as evidenced by an increased nucleotide substitution rate in the cytochrome *b* gene as compared to the *zfy* gene. The cytochrome *b* gene appears to be evolving at ten times the rate of the cytochrome *b* gene as can be seen in the linear portion of the curve.

The plot in figure 17 also provides information as to the level of saturation present in the cytochrome *b* gene as compared with the *zfy* gene. At around 13% - 15% the cytochrome *b* gene becomes saturated while the *zfy* gene has not reached saturation. This has implications for the utility of the cytochrome *b* gene for phylogenetic inference at levels of divergence as found in *Scotophilus*. The cytochrome *b* gene is best suited for comparisons, where saturation is less likely to be a problem and should be used cautiously at high levels of divergence. Even in this study, the cytochrome *b* gene is totally saturated above the 12% - 15% level of divergence.

Plots in figures 15 and 16 show the level of saturation seen in the cytochrome *b* gene. Figure 15 shows the individual codon position plots and a characteristic result is

seen. First position comparisons of corrected versus uncorrected distances are linear and second position changes appear rare. Therefore, neither of the positions reveal saturation effects. The plot for 3rd positions shows evidence of saturation, thus potentially reducing the phylogenetic signal. Figure 16 shows the plot for the entire cytochrome *b* gene as well as a plot of the positional contributions of each of these three codon positions to the plot of the entire cytochrome *b* gene.

The Mammalian Y Chromosome as a Phylogenetic Tool

The mammalian Y chromosome has been previously proven as an effective tool for understanding human population origins and gene flow (Jobling and Tyler-Smith, 2003; Semino et al., 2002; Bosch et al., 2001). Previous studies on *Equus* (Wallner et al., 2003) and the family Felidae (Slattery and O'Brien, 1998) demonstrated the use of the Y chromosome in a phylogenetic context. This study on *Scotophilus* further lends support to the use of Y chromosome markers in inferring species phylogenies. The Y chromosome was shown to accurately define species groups and the phylogenetic relationships corroborated those relationships seen with the more traditional mitochondrial cytochrome *b* marker. In theory, the Y chromosome should more closely reflect phylogenetic history as the Y chromosome is a subset of the nuclear genome and its evolutionary trajectory is tied to that of nuclear genes. The Y chromosome also provides an interesting point of view on evolution, the male perspective, as it is inherited from father to son in a clonal fashion. Studies on well supported phylogenies that are known to be accurate will help bolster the case for Y chromosome phylogenies. Slattery and O'Brien (1998) showed that sequences of an intron of the *zfy* gene accurately

tracked hierarchical feline topologies. They noted that the Y chromosome demonstrates a "remarkable degree of phylogenetic consistency", and concluded that *zfy* is "highly accurate in recapitulating evolutionary history". This study of *Scotophilus* demonstrates that the Y chromosome is useful at recovering species level phylogenies, in defining species, and in comparisons to mtDNA derived phylogenies. The utility of the Y chromosome for the inference of phylogenies has been demonstrated. The Y chromosome provides an opportunity to evaluate mtDNA derived phylogenies and to more appropriately define species based on a nuclear gene along with the more traditional mtDNA genes currently being employed.

CONCLUSIONS

The genus *Scotophilus* continues to be a difficult genus of bats to characterize and the definition of the phylogenetic relationships among some members of the genus remain unresolved. The results of this study provide evidence for two additional species of *Scotophilus* based on cytochrome *b* and *zfy* data and confirmation of two other morphologically described species. Molecular support for a newly described Malagasy species as well as for *S. nigritellus* is demonstrated. These four species are: *S. sp. nov.* (Goodman et al., in press); *S. nigritellus*; *S. viridis* (eastern Africa); and *S. dinganii* (eastern Africa). The total number of *Scotophilus* species known including these four is now 16.

The systematic conclusions drawn from this study include: *S. kuhlii* as the most basal taxon, monophyly of African *Scotophilus* with *S. nux* as the most basal African taxon, a distant relationship between the two Indomalayan species (*S. kuhlii* and *S. heathi*) suggesting multiple origins of Asian *Scotophilus*, Malagasy *Scotophilus* appear to have affinities with the African species and likely multiple origins from Africa as *S. robustus* and *S. sp.* do not share a sister group relationship, *S. nigrita* appears as closely related to *S. dinganii* and may have hybridized with a *S. dinganii*-like ancestor, and the *S. dinganii*-like species all share a close relationship.

The use of the Y chromosome as a phylogenetic tool has been demonstrated in this study to effectively assist in phylogeny reconstruction and the elucidation of systematic relationships. The Y chromosome provides a good contrast to mtDNA markers and in conjunction with the more traditional mtDNA markers provides a more

thorough view of the evolutionary history of the taxa in question. Confirmation of these newly described species by traditional morphological techniques would further support these taxonomic conclusions.

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APPENDIX A

In this study I sequenced the cytochrome *b* gene for *S. heathi* (MVZ 176513) from China, Yunnan Province and also obtained a cytochrome *b* sequence for this same individual submitted to GenBank (GenBank Accession AF376831) that was sequenced by Ruedi and Mayer (2001). My nucleotide sequence obtained from this individual differs from that obtained through GenBank at ten nucleotide positions. The differences in the sequence are shown in figure 15 below and range from nucleotide position 784 to the last difference at nucleotide position 1122. The chromatogram tracings for each nucleotide position that differs are shown in figure 15. The chromatogram tracings demonstrate that the nucleotides called are accurate as there is no background noise or other peaks interfering with the quality of the sequence. The difference between my sequence and the genbank sequence is 0.8%. In the context that Ruedi and Mayer (2001) used the sequence, the difference should have minimal impact on the branching order of their ingroup taxa (genus *Myotis*). This difference though would be substantial in an intra-species population genetics study and could potentially be very misleading. The differences in the two sequences are located in the downstream portion of the cytochrome *b* gene and thus all probably are the result of a single sequencing extension (sequencing primer). This instance makes a strong argument for careful quality control of sequences and sequencing of both strands when necessary to confirm noisy sequence data.

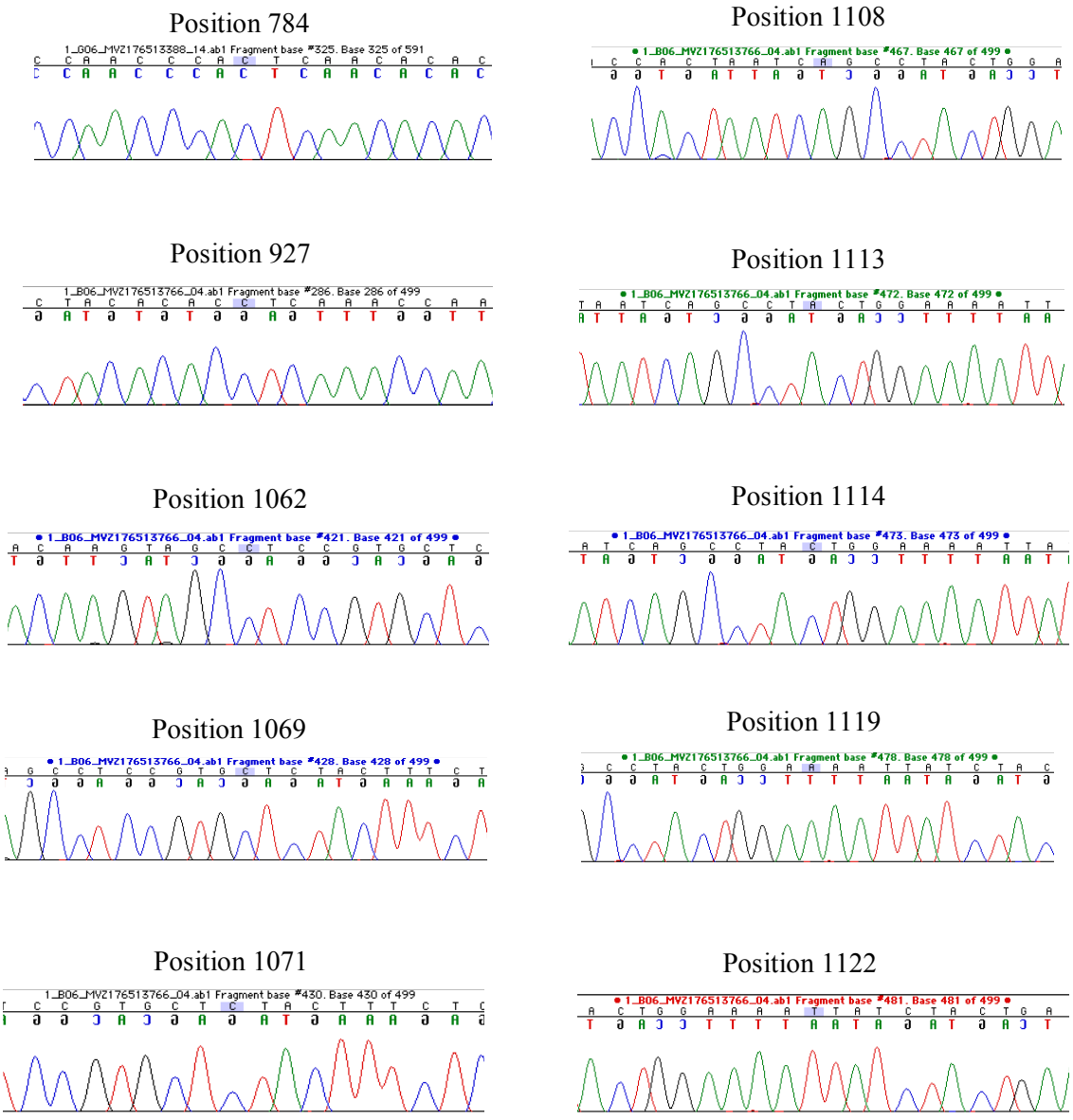

Figure 18. The ten nucleotide positions in the cytochrome *b* sequence of *S. heathii* MVZ 176513 that differ from the GenBank sequence for the same individual specimen. The nucleotides are highlighted for each position on the chromatogram tracing.

APPENDIX B

The individual specimens in each clade of the phylogenies in figures 3 – 7 are listed by clade number and from top to bottom within each clade. They are as follows: **Clade 1** = AK 21476, AK 21479, AK 21481, AK 21485, AK 21482, ROM 110842, ROM 110956, ROM 110837, ROM 110957, AK 21489, ROM 110840, MVZ 186421, MVZ 186418, EAR 1266, ROM 110843, AK 21477, ROM 110841, AK 21484, ROM 110835; **Clade 2** = SP 5071, SP 5055, TK 33485, SP 5054, SP 5053, SP 10620, SP 10628, SP 10629; **Clade 3** = SP 10136; **Clade 4** = RBJ 161, RBJ 215, SMG 14474; **Clade 5** = MVZ 176513 GenBank sequence, MVZ 176513, ROM 107786, MVZ 186412; **Clade 6** = FMNH 166186, FMNH 151939; **Clade 7** = TM 37691, TM 37672, TM 38149, TM 37675, TM 39482; **Clade 8** = SP 11111, SP 11112, SP 11050, SP 11041, SP 10161; **Clade 9** = SP 7731, SP 7789, TM 39625, TM 37669, TM 37668, F 52131, SP 7732, SP 7755; **Clade 10** = SP 5498, SP 5500, SP 5499, TK 33266, TK 33264, TK 33265; **Clade 11** = SP 10179, SP 10180, SP 10181; **Clade 12** = SP 5051, SP 5077, SP 5078, TK 33536, TK 33534, SP 5052; **Clade 13** = AK 21213, SP 5505, AK 21223, AK 21259, SP 13027; **Clade 14** = SP 5451, SP 5370, SP 5369, TK 33149, TK 33142, TK 33360, TK 33395, TK 333189, SP 5454, SP 5452; and **Clade 15** = AK 21163, SP 5368.

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